

ANNUAL REPORT

**Division of Intramural Research Programs
National Institute of Mental Health**

October 1, 1986 - September 30, 1987

**VOLUME II PART 1
INDIVIDUAL PROJECT REPORTS**

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Alcohol, Drug Abuse, and Mental Health Administration
National Institute of Mental Health
Division of Intramural Research Programs**

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ANNUAL REPORT

DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1986 - September 30, 1987

VOLUME II PART I

INDIVIDUAL PROJECT REPORTS

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ANNUAL REPORT
DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1986 - September 30, 1987

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DIVISION OF INTRAMURAL RESEARCH PROGRAMS
NATIONAL INSTITUTE OF MENTAL HEALTH

RESEARCH PROJECT SERIAL NUMBER LISTING:

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02188-03 OCD

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Studies of Borderline Personality Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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	D.L. Murphy	Chief	LCS, NIMH

COOPERATING UNITS (if any)

Office of the Director, Division of Intramural Research Programs, NIMH;
 Biological Psychiatry Branch, NIMH; Laboratory of Clinical Science, NIMH

LAB/BRANCH

Office of the Clinical Director

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

1.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patients with borderline personality disorder and rejection-sensitive dysphoria participated in a program of clinical and biological evaluation. In addition to labile moods and behavioral dyscontrol, a high incidence of discrete major depressive episodes has been observed. Mood ratings recorded twice daily on visual analogue scales by borderline patients were compared with ratings completed by patients with major depression, patients with premenstrual syndrome and normal volunteers and revealed distinguishing patterns between the four groups with the borderline patients showing low global mood ratings with high variability.

On standardized psychiatric rating scales, borderline patients scored high on depression (Beck Depression Inventory), cognitive symptoms of depression (Dysfunctional Attitude Scale) and hostility (Buss-Durkee Hostility Aggression Index) even when not in a current depressive episode. Neuropsychological testing revealed a pattern of poor performance in tests of tonal memory, a function linked to the right temporal lobe, and in tests measuring visual-perceptual abilities.

Computerized tomography scans of the brain were studied and compared to scans of healthy normal volunteers. No significant differences were found between the two groups on measures of ventricular brain ratio, third ventricular size or evidence of frontal lobe atrophy. Lumbar puncture procedures are being performed to measure cerebrospinal fluid metabolites. Naloxone infusions are being performed to investigate alteration in pain mechanisms. Preliminary analysis reveals elevated baseline levels of beta-endorphin and ACTH in the borderline patients when compared to normal volunteers.

The serotonin agonist, m-chlorphenylpiperazine (m-CPP), was associated with an activated, euphoric response and preliminary results suggest a blunting of the prolactin response. Noise and learning, a paradigm of performance under stress modeled after the learned helplessness model, is being administered to study reactions in stressful situations and associated neuroendocrine responses.

PROJECT DESCRIPTION

Rejection-sensitive or hysteroid dysphoria is a poorly understood syndrome occurring in many individuals with a diagnosis of borderline personality disorder. This syndrome, described by Klein and others, is characterized by the rapid onset of a dysphoric mood (sometimes characterized more specifically by depression, anxiety, or rage) following an actual, threatened, or imagined rejection. Behavioral dyscontrol is not uncommon, involving violence, direct injury to self, or overdosage with sedating medications. This disorder accounts for a significant number of admissions to short-term psychiatric units, and is one of the more difficult disorders treated in long-term outpatient psychotherapy.

There are a number of theories about the etiology of the borderline personality in general, and rejection-sensitive dysphoria in particular, most emphasizing developmental psychodynamics. However, recent phenomenological and family history studies of the disorder suggest that this disorder may represent a variant of affective disorder, a neurophysiological dysfunction of limbic system functioning, or in some cases an adult variant of minimal brain dysfunction. To date, little biomedical research has been done to explore possible underpinnings of this disorder.

Previous findings in this project tend to support a role for biological factors in the various symptoms of this disorder. A high prevalence of neurological soft sign abnormalities and psychomotor-psychosensory symptoms were found in this population. Results of medication trials showed that carbamazepine, an anticonvulsant was effective in reducing episodes of dyscontrol and impulsivity, while tranlycypromine proved to be an effective antidepressant with some reduction in emotional lability. Alprazolam, an antianxiety agent, lessened anxiety but was associated with increased impulsivity and behavioral dyscontrol.

This project continues to explore the relationships among clinical phenomenology, developmental factors, and family histories; neurophysiological function (neuropsychological testing); and biochemical measures including cerebrospinal fluid studies, provocative infusion studies (naloxone, m-chlorphenylpiperazine) and endocrine challenge tests (TRH stimulation tests and dexamethasone suppression tests).

MAJOR FINDINGSPhenomenology

In addition to elaboration of dysphoric episodes and self-injurious behavior described in the previous annual report, we have focused on descriptions of affective symptomatology and perceptual abnormalities. Depression symptoms generally are characterized by marked mood lability, often unrelated to environmental or psychological stimuli; however, a high incidence of discrete major depressive episodes has also been observed. Along with a high family incidence of depressive disorders, this suggests a strong connection between the borderline disorder and affective disorders.

Daily Ratings

Mood lability was further explored with self-ratings of mood recorded twice daily using visual analogue scales. These ratings reflect global mood as well as the variability of mood during a given day and variability of mood from day to day. Ratings by borderline patients were compared with patients with major depression, patients with premenstrual syndrome, and normal volunteers. Borderline patients showed low global mood ratings and a high degree of diurnal variation and day to day variability, and the combination of these two factors differentiated them statistically from patients seen in the other three groups.

Neuropsychological Testing

Preliminary results of neuropsychological testing completed on 14 borderline patients and 11 normal volunteers, show a pattern of poor performance by the borderline patients on the Seashore Test of Musical Talent, a measure of tonal memory usually linked to functions of the right temporal lobe, and poor performance on visual perceptual tasks. Patients with borderline personality disorder scored high on the Beck Depression Inventory and the Dysfunctional Attitude Scale, measures of depressive symptoms and cognitions related to depression. Patients also rated themselves high on the Buss-Durkee Hostility Aggression Index, a measure of anger and hostility. Information is being gathered on dissociative phenomena using the Dissociative Episode Scale, and phenomena related to temporal lobe abnormalities using the Bear-Fedio Inventory and the psychomotor/psychosensory symptom rating scale.

Biomedical Studies

To examine systematically the various neurotransmitter systems in the borderline personality disorder, we planned a series of provocative infusions which have been performed in other psychiatric populations (e.g., schizophrenia, major depression). Because of the altered pain mechanisms in this disorder, we administered naloxone, an opiate antagonist, in a dosage of 2 mg/kg, to nine borderline patients and 11 normal volunteers. Clinically, one borderline patient reacted with a transient depressive response and one normal volunteer described feeling "out of sorts" and "down" for several hours. Preliminary results of biochemical studies shows a higher baseline level of beta-endorphins and ACTH in the borderline patients than in the normal volunteers. Naloxone appears to have a negligible effect on endorphin levels.

The serotonin agonist, m-chlorophenylpiperazine (m-CPP), was administered to examine the effects of activation of the serotonergic system on symptomatology. Seven borderline patients received oral doses of .5 mg/kg. Five patients had activated responses, including 2 euphoric responses, 2 happy/giddy responses, and one energized/sarcastic response. Two patients had no clinical response. Preliminary results of biochemical studies suggest a blunting of the prolactin response when compared with studies in normal volunteers. Further studies are planned.

Cerebrospinal spinal fluid samples have been collected via lumbar puncture from nine borderline patients. These are being assayed for neurotransmitter metabolites and will be compared with samples from normal volunteers.

Noise and learning, a behavioral paradigm based on the learned helplessness model of depression, examines the performance of patients under stressful conditions and blood samples measure hormonal responses to stress. Seven borderline patients have participated in this procedure thus far. Two patients terminated the procedure before completion, stating the procedure was too stressful. Five patients completed the procedure. Preliminary analysis of hormonal response suggests an exaggerated cortisol response when compared with normal controls. Further studies will be pursued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

Rejection-sensitive dysphoria and borderline personality disorder are common disorders, particularly in the young adult population. They account for a significant number of short-term psychiatric hospitalizations and are frequently associated with major, often life-threatening overdoses, with self-mutilation, and with episodes of violence. The etiology of these disorders is a matter of great controversy and limited data, as is the role of medication in the treatment of these individuals.

The evaluation phase of this study provides tentative support for a theory of these disorders which emphasizes an interaction between developmental traumata and biological predisposition. If further studies confirm the association between low threshold for dysphoria and dyscontrol on the one hand and procaine induced high frequency EEG activity over the temporal lobe on the other, the line between limbic system abnormalities and labile mood and impulsive behavior is strengthened. Specific pharmacologic strategies for altering the responsivity of limbic system structures may ameliorate the dysphorias, may lessen the likelihood of dyscontrol, and may enhance the usefulness of psychotherapy in this disorder.

PROPOSED COURSE

Further clinical, developmental, and biological data are needed from a larger number of patients and normal volunteers. We plan to continue the studies discussed in the report through the upcoming year. In addition, neuroanatomical and neurophysiological issues will be addressed by expanding some of these procedures. We will explore differences in brain structure through VBR measurements in borderline patients as compared to normals and schizophrenic patients. Xenon blood flow, SPECT, or PET methodologies will be coupled with procaine-activation of dysphoria in an attempt to identify brain structures involved in dysphoric process.

PUBLICATIONS

Cowdry RW, Gardner DL: Pharmacotherapy of borderline personality disorder: alprazolam, carbamazepine, trifluoperazine, and tranylcypromine. Archives of General Psychiatry. (in press).

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Lucas PB, Gardner DL, Wolkowitz OM, Tucker EE, Cowdry RW: Methylphenidate-induced cardiac arrhythmias. New England Journal of Medicine, 315(23): 1485, 1986.

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Cowdry, RW: Psychopharmacology of borderline personality disorders: a review. J Clin Psychiatry, in press.

Gardner DL, Cowdry RW: Development of melancholia during carbamazepine treatment in borderline personality disorder. J Clin Psychopharmacol 6(4):236-239, 1986.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00183-02 BP

PERIOD COVERED

October 1, 1986 - September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biology and behavior of aggression and suicide

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Gerald L. Brown, M.D., Medical Officer, BPB, NIMH

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 M. Linnoila, M.D., Chief, LCS, NIAAA; J. Kleinman, M.D., St. Elizabeth's Hospital,
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COOPERATING UNITS (if any)

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INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.6

OTHER:

.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

For several years, studies that relate human aggression (including hyperactivity and conduct disorder in children) and suicide to various behavioral and biological factors have been ongoing. Some of the most significant findings have included pharmacokinetic and metabolic studies of amphetamine administered to hyperactive and conduct disordered children, and a trivariate relationship among a history of aggressive behavior, a history of suicidal behavior, and lower cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5HIAA). More recently, data indicate that certain aggressive, impulsive, and depressive characteristics in childhood are also inversely related to CSF 5HIAA measured during late adolescence; family instability during childhood also appears to be associated with increased likelihood of aggressive and/or suicidal behavior in late adolescence. These data, along with the work of other investigators studying aggressive behavior in childhood, indicate the possibility of traits associated with disordered serotonin metabolism; further, the less consistent relationship between lower CSF 5HIAA and suicidal behaviors vs. aggressive behaviors, may indicate that some suicidal behaviors are a self-destructive manifestation of a more basic destructive (aggressive/impulsive) trait.

Project Description:

Objectives: An objective is new knowledge of the central nervous system (CNS) of children, adolescents, and adults with special reference to maturational changes and neuropsychiatric disorders as they relate to aggression and suicide. Compared to neurobiology known in adult neuropsychiatry, less is known regarding neuropsychiatric disorders of children. There have been a number of hypotheses relating catecholamine metabolism and hyperactivity in children. The possibility of an overly active catecholaminergic system was first advanced. Later, a functional deficiency in catecholamines in hyperactive children (now subsumed under DSM-III diagnoses, Attention Deficit Disorder [ADD] and Conduct Disorder [CD]) was proposed, with the greater focus on the possibility of a functional dopamine (DA) rather than norepinephrine (NE) deficiency. Other biochemical alterations, particularly involving serotonin (5HT), have also been proposed. More recently, alterations in phenylethylamine (PEA) have also been proposed. No single neurotransmitter system to date can be shown to have an etiological role. Several new populations of children and adolescents are now being studied; i.e., aggressive CD, obsessive-compulsive disorder (OCD), children who have been abused, and those with multiple personality disorders (MPD).

Considerable indirect pharmacologic evidence has linked amine systems with adult psychiatric illness (particularly affective illness and schizophrenia). Searching for interrelationships between central biochemical functioning and repeated behavioral patterns may be as important as searching for traditional diagnostic specificity of biochemical findings. Confirmation of relationships between central biochemistry and behavior could lead to more specific pharmacological treatments. Direct human data can be valuable in utilizing animal data and in assessing the differences and similarities between man and animals. Data has begun to be accumulated on central neurochemical function in the various personality disorders; i.e., the "aggressive-impulsive" personalities and OCD's. Personality disorders involving criminality show indications of a genetic component; aggressive/impulsive characteristics have also been linked to a genetic predeterminant of suicidal behavior independent of psychiatric diagnosis. Furthermore, other patterns of behavior often seen within personality disorders - depression, alcoholism, suicide, and obsessiveness - also appear to have genetic components. Data from animals strongly suggest a relationship between aggressive behavior and neurotransmitters. A purpose of this project is to extend the studies of central amines into larger and more diverse populations of psychiatric patients, and to assess behavioral-biochemical relationships and whether such findings are diagnostically specific. Dr. Frederick Goodwin continues to provide overall scientific supervision of this multi-faceted project.

Methods Employed: An inpatient and day-patient program for children and adolescents, involving selected overnight stays, is ongoing on an inpatient nursing unit. Children and adolescents who are hyperactive, aggressive/impulsive, and obsessive are admitted in order to study a carefully defined sample of ADD/CD and OD. Specific exclusion and inclusion criteria are employed. All children are thoroughly evaluated by medical, psychiatric, and psychometric examinations with all routine and other indicated procedures and clinical laboratory studies. Several clinical and behavioral rating instruments are utilized. Pharmacological study results implemented in this program are briefly summarized below. Further details of this program can be found in "Cerebrospinal Fluid in Childhood

Behavioral Disorders", Protocol #85-M-115 of Dr. Judith Rapoport. The studies done in collaboration with Dr. Frank Putnam on children and adolescents with multiple personalities; often aggressive/impulsive and the victims of child abuse; have been delayed, but are still anticipated.

Previous NIMH-Navy studies have been described in detail in a previous annual report (Z01 MH 00092-11 BP). The results of these studies are briefly summarized below.

Currently, clinical studies are being carried out in subjects with several kinds of disorders. Of particular clinical interest is the interrelationship between aggression and suicide. These subjects will have their indoleamine metabolism assessed directly and indirectly in several ways; i.e., cerebrospinal fluid (CSF) obtained by lumbar puncture (LP), response to glucose tolerance testing (GTT) in alcoholics. Family studies are under way in these subjects as well (Dr. Linnoila). LP's are being performed in children and adolescents who are aggressive/impulsive or compulsive (Dr. Kreusi). Blind clinical evaluations are being performed by Dr. Brown.

Among those individuals incarcerated for murder, responses to GTT and similar artificial sweetening will be assessed by the Thematic Apperception Test (TAT) along with baseline LP's in collaboration with Dr. Linnoila and colleagues. This project has recently been approved by an HHS Ethics Committee. A further study involves an assessment of serotonin and its metabolite from autopsy material and LP's in conjunction with their clinical inpatient records in those individuals with a history of suicide and/or violence (in collaboration with Dr. Kleinman). Aggressive/impulsive behavior is also being assessed in adult patients with affective illness, obsessive-compulsive disorder, and normals (Dr. Murphy).

Major Past Findings: Serial plasma pharmacokinetic data indicate that d-amphetamine (d-AMPH) reaches a peak level in children with ADD/CD within 3-4 hours of an initial dose; however, as much as 70-80% will remain in the serum at 5-6 h when behavioral effects have largely dissipated. Mean apparent elimination half-life is 6.8 ± 0.5 h. Test-retest studies of individuals indicate that both pharmacokinetic data and clinical response data are highly replicable. Sustained release capsules produce a slower rate of absorption and a more plateau-like, longer-lasting peak level, but do not give a prolonged clinical response. Socially appropriate behavioral change and motor activity decrease are maximal at 1-3 h after administration of a single dose (0.5 mg/kg) of d-AMPH. Higher single doses (1.0 mg/kg) effect earlier similar clinical response, but of less magnitude. Piribedil is safe but clinically ineffective in ADD/CD. d-AMPH has been shown to have an anti-aggressive effect in aggressive/impulsive children. Preliminary results indicate that neither tryptophan (TP) nor valine (a neutral amino acid which competes with TP and inhibits its crossing the blood-brain barrier) results in behavioral response or basal temperature change after a single dose, but attention span increase is similar to that observed following d-AMPH, while there are clear effects on plasma amino acids in the expected directions. Another preliminary study indicates that both plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) and homovanillic acid (HVA) are affected acutely by single-dose d-AMPH in a non-pretreated child.

Urine studies in ADD/CD indicate that day and night excretion of MHPG and HVA are not different; however, d-AMPH after 8 and 14 days is associated with lower MHPG levels. Behavioral response may be associated with the decrement in MHPG. Urinary HVA is unchanged. Tyramine (TRM) and parahydroxyphenylacetic acid (PHPA) excretion are also decreased and phenylethylamine (PEA) excretion is markedly elevated following two weeks of d-AMPH. PEA excretion is lower in ADD/CD versus controls; its significance depends on whether it is expressed in terms of creatinine excretion. More recent studies indicate a different pattern of metabolite response to methylphenidate (MP). Both d-AMPH and MP effect no change in DA or its metabolites.

ADD/CD are not different from normals with regard to plasma NE and dopamine-beta-hydroxylase (DBH) but do have significantly more neurological soft signs by PANESS examination. New item analysis data indicates the prevalence of varied soft signs and their rater reliability. Plasma NE correlates with anxiety ratings and changes both with regard to dose of d-AMPH and time following dose, with higher doses of d-AMPH (1.0 mg/kg) giving strongest response at 1 hour and lower doses (0.5 mg/kg) giving strongest response at 3 hours. Elevated plasma NE is also associated with increases in blood pressure and pulse, and is dose-related. Baseline plasma NE, measured prior to an early a.m. dose of d-AMPH, does not change after two weeks of d-AMPH versus two weeks of placebo.

With regard to pharmacological response, d-AMPH is effective and piribedil and L-DOPA are minimally so; TP produces responses similar to d-AMPH. ADD/CD, with higher levels of soft signs, have more abnormal EEG's, more minor physical anomalies, lower full-scale I.Q.'s (WISC-R), and a greater number of errors on the Bender. Data from psycholinguistic evaluations indicates that ADD/CD have impairments in certain auditory processing and language skills; furthermore, d-AMPH does not evoke pronounced effects with regard to language performance in ADD/CD vs. normals; older and less hyperactive subjects showed the most improvement. Improvement in cognitive parameters was shown only in normals.

Initial results from late adolescent personality disorders with problems secondary to poor impulse control, high levels of anger-hostility, and poor judgment indicated that aggressive behavior is inversely correlated with CSF 5-hydroxyindoleacetic acid (5HIAA) and positively correlated with CSF MHPG. Personality disorders have shown no significant difference in CSF cyclic 3',5-adenosine monophosphate (c-AMP) from neurological patients with non-CNS disorders or from depressive, manic, and schizophrenic patients. Aggressive behavior was positively correlated with c-AMP and cyclic-3', 5-guanosine monophosphate (c-GMP) in one group but not in a second. Those who were administratively discharged from the Service and those with history of suicidal attempts had lower CSF 5HIAA and higher MHPG, c-AMP, and c-GMP. Borderline personalities (DSM-III) in a second study showed an inverse relationship between CSF 5HIAA and the psychopathic deviate (Pd) (MMPI) scale, as well as a history of aggressive behavior; neither the MHPG relationships nor the cyclic nucleotide relationships were replicated. A trivariant relationship among a history of aggression, history of suicidal behavior, and lower CSF 5HIAA is apparent.

As experience accumulates from various collaborative studies, the aggressive variable that appears to be most likely associated with lower CSF 5HIAA is that characterized by lability of affect, history of repeated impulsivity, and explosiveness. Similarly, our experience and that of others appears to indicate that suicidality

associated with aggressivity is most likely to be associated with reduced levels of CSF 5HIAA.

A military male found guilty of violent murder, with a past history of several suicidal attempts, was found to have the lowest level of CSF 5HIAA yet measured by our group; he also had a hypoglycemic response to a GTT. In that aggressive behavior has been shown in animals to be associated with lower GABA, new studies of CSF GABA, both free and bound, have been analyzed in the borderline group of patients; though CSF GABA is lower in the more aggressive patients and in those with histories of suicidal behavior, neither difference reaches the $<.05$ level of significance.

Alcoholics do not differ from personality disorders in CSF HVA. However, mean CSF 5HIAA is higher in the intoxication-withdrawal stage and decreases over time in abstinence to reach a mean level not differing from that of personality disorders. CSF HVA levels are depressed by disulfiram (Antabuse), a DBH inhibitor. Disulfiram use also correlates with an increase in serum NE. Mean serum DBH in alcoholics versus normal controls was lower, blood pressure was higher, and serum NE was not different. Disulfiram is also associated with an increase in cholesterol in alcoholics. Lower CSF DBH is correlated with increasing psychopathology, as measured by the MMPI, and lower CSF DBH is associated with disulfiram-induced psychoses. Furthermore, low platelet monoamine oxidase (MAO), low amine oxidase (AO), and elevated erythrocyte catechol-0-methyltransferase (COMT) are associated with disulfiram-induced psychoses. Neither clinical depression nor aggressive behavior in this group of early to mid-stage alcoholics can be associated with alcoholism; nor can improvement in depression or anxiety ratings of hospitalized alcoholics be attributed to disulfiram.

Further analyses of previous studies indicate that those individuals diagnosed as antisocial and explosive (DSM-III) have the lowest levels of CSF 5HIAA. Furthermore, the MMPI profile of 42, 28, and 49 with high F scale scores is most closely associated with low CSF 5HIAA. The only Buss-Durkee Inventory (BDI) category that has a significant inverse relationship with CSF 5HIAA is "irritability". While total BDI scores and PD T scores do correlate significantly with a life history of aggression, the BDI appears to measure a number of aspects of aggressive thoughts and attitudes as well as behavior, but this scale appears to be a less useful instrument to relate to CSF 5HIAA levels.

Platelet MAO is not significantly different in medication-free ADD/CD vs. normals; AO is significantly lower in ADD/CD vs. normals. MAO was not correlated with age in normal children (groups not different with age as a covariate); AO was not correlated with age in either group. MAO and AO levels were not related to a low monoamine diet platelet number, Hgb and Hct did not differ in the groups, nor was MAO or AO correlated with either. MAO and AO were determined two times in 20 subjects; the percentage coefficients of variation (CV) were 18.6 ± 9.4 and 12.0 ± 9.2 , respectively. Finally, neither MAO nor AO responds significantly to d-AMPH.

Platelet 5HT, though not different in ADD/CD vs. normals is negatively correlated with both attentional and conduct factors on the Connors Teachers-Rating Scale (CTRS), more strongly with conduct. These findings may explain the discrepant reports of 5HT in ADD/CD when group data is compared to normals.

A very low level of CSF 5HIAA was found in a conduct-disordered adolescent, whose stealing involved a craving for sugar (glucose intake increases brain levels of 5HT). This individual also had an MMPI profile similar to that reported by Brown and colleagues for aggressive male adolescents with low levels of CSF 5HIAA.

New Finding: As part of Dr. Brown's collaborative role in making clinical assessments of aggressivity-impulsivity in children and adolescents, in collaboration with Drs. Kreusi and Rapoport, he remains blind to CSF 5HIAA data that has now been collected on approximately 20 such subjects. Further refinements in both the content and reliability of intra-rater assessments are ongoing as the study continues. Finding that a negative correlation exists between CSF 5HIAA (in the later group of Navy men) and a childhood history of ADD/CD kinds of behavior has been furthered. Medical history includes headaches in childhood. These findings have been further pursued in terms of suicidal behavior and family history of various kinds of stresses and instability. Data have now been analyzed that show that both those individuals with a higher score for aggressive/impulsive behaviors, and those who have a history of suicide attempts, had greater mean scores on items related to family instability, stress, and loss than those with lower scores for aggressive/impulsive behavior and those without a history of suicidal attempt. Of interest is that disturbed family history per se is not related to levels of CSF-5HIAA, possibly indicating that various kinds of disturbed personality and behavior (present in all of the subjects whom Dr. Brown has studied) not of an aggressive and/or suicidal character, may also not be associated with serotonin (5HT) metabolism, or at least not in the same way as that seen for aggressive/impulsive/suicidal individuals. These findings are consistent with other data in the literature that would support an inverse relationship between aggressive/impulsive behavior or behavior thought to indicate dyscontrol and disinhibition as trait characteristics and CSF 5HIAA or other measures related to 5HT metabolism. The review of histories of schizophrenics for aggressive/suicidal behavior with LP and autopsy material is ongoing. Collaborative studies with Dr. Murphy have just begun.

Significance to Mental Health Research: Though childhood neuropsychiatric disorders have been considerably studied in the last few years, many diagnostic, psychopharmacological, and psychobiological questions are yet to be addressed; an increased interest in psychopharmacology continues to emerge. Though MP and AMPH give positive responses in 80% of well diagnosed ADD children, the pharmacokinetics and metabolism of these drugs have been studied only relatively recently. Drug responsiveness in CD is less clear. One avenue to ascertaining possible neuropathology is to understand more clearly the mechanisms of action of pharmacological compounds which effectively alter the clinical conditions under study. The relationship between such basic pharmacological knowledge and clinical effects has been under-studied in children in general. More importantly, for the future, basic biological factors in childhood neuropsychiatry which might elucidate the psychopharmacological responses are, at this point, only hypotheses. The degree to which these hypotheses are validated or refuted could be expected to play a significant role in understanding childhood neuropsychiatry.

CNS functioning appears to have been under-studied in some major groups of psychiatric patients; viz., personality disorders, alcoholics, and borderlines. Studies of animal models, as well as Gilles de la Tourette's syndrome, ADD/CD in children, and prisoners suggest a relationship between central neurotransmitter systems and

aggressive behavior. Human suicidal behavior has an enormous public health and social significance and, previously, had largely been studied from psychological and sociological points of view. Both suicidal and aggressive problems are increasing. These studies lead to the possibility of identifying those at risk for anti-social and suicidal behaviors and possibly altering those behaviors through neuropharmacological adjuncts to management of the psychiatric and/or behavioral problems. The neurobiological aspects of alcoholism, either predisposing, concomitant, or resultant, are also of timely significance.

Proposed Course of Project: The principal investigator, Dr. Brown, remains in the Office of the Chief, BPB, and is no longer administratively a part of the Child Psychiatry Branch of the Intramural Research Program (IRP), although active collaboration continues with this Branch as well as the Laboratory of Developmental Psychology (Putnam), and Dr. Brown serves as a special consultant to the Child Psychiatry Branch in the Extramural Program (ERP). There is a body of data yet to be analyzed but some of this is in preparation and in press and will be reported in the future.

The preparation for the Navy collaborative project began in January 1973. The approval processes, both in terms of scientific merit and the protection of rights of patients, were completed in July 1974. Though the active data collection phase of the collaboration has been terminated, this collaboration continues to be of mutual benefit to NIMH and NNMC. Some neurochemical, behavioral, and psychological data are yet to be analyzed and reported from the patients who have participated in these studies, as well as the attempts at follow-up. Additionally, collaboration, though delayed by other duties, continues on LP and autopsy studies of schizophrenics within the IRP at St. Elizabeth's Hospital.

PUBLICATIONS:

Brown, G.L. and Goodwin, F.K.: Human aggression and suicide. In Maris, R. (Ed.): Biological Aspects of Suicide, Vol. 16. New York, Guilford Press, 1986, pp. 141-161.

Brown, G.L. and Goodwin, F.K.: Cerebrospinal fluid correlates of suicide attempts and aggression. In Stanley, M. and Mann, J. (Eds.): Psychobiology of Suicidal Behavior, Ann. NY Acad. Sci. 487: 175-188, 1987.

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Brown, G.L. and Goodwin, F.K.: Risk factors in suicidal behavior. Psychiatry Lett., in press.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00070-14 BP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychological and Biological Interactions in the Mood and Anxiety Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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TOTAL MAN-YEARS:

13.0

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7.0

OTHER:

6.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Evaluation, study, and treatment of patients with manic-depressive and schizoaffective illness are the primary goals of the Section. Double-blind, placebo-controlled clinical trials are employed to evaluate routinely used and novel agents for the treatment of these disorders. Anticonvulsants such as carbamazepine have been demonstrated to be clinically effective in the acute and prophylactic treatment of manic-depressive illness. We have identified possible clinical and biochemical markers of response to lithium versus carbamazepine and other agents. For example, antimanic responders to carbamazepine appear to be more severely ill, more dysphoric, and more rapidly cycling than non-responders, i.e., variables that tend to be associated with lithium nonresponse. In attempting to elucidate possible mechanisms of action, we have found that alpha-2 noradrenergic and "peripheral-type" benzodiazepine receptor mechanisms may be important to the anticonvulsant if not the psychotropic effects of carbamazepine. Other neurotransmitter, modulator, and peptide substances are being studied which may account for carbamazepine's positive effects on mood and behavior. The Section also seeks to identify regional alterations in brain electrophysiological and metabolic activity that are related to changes in behavior and cognition in affective illness. A clinical probe of limbic system excitability utilizing a novel provocative agent, procaine, is also being employed. Procaine selectively increases fast activity over the temporal lobe in association with a variety of behavioral and cognitive alterations and secretion of cortisol, ACTH, and prolactin. Animal models of electrophysiological and pharmacological kindling and cocaine-induced behavioral sensitization are studied and implicate conditioning and learning processes in the progressive behavioral changes induced. These models may help provide new clinical and biochemical insights into the mechanisms that underlie the progressive and long-term changes in behavior in a variety of clinical syndromes including cocaine-induced psychopathology and affective illness.

COLLABORATORS:

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I. Project Description

A. Objectives

This project is engaged in the multidisciplinary longitudinal study and treatment of patients with a spectrum of acute and chronic psychoses, particularly involving mood and anxiety disorders. Both investigative and treatment approaches focus on the elucidation of psychological and biological phenomena and their interaction.

B. Methods Employed

1. Subjects who meet Research Diagnostic Criteria (RDC) for manic-depressive or schizoaffective illness or the more recent DSM III criteria for a spectrum of mood disorders are admitted to the 3-West Clinical Research Unit, Section on Psychobiology of the Biological Psychiatry Branch. Patients with anxiety and panic-anxiety are also admitted to the unit under other protocols (see Project Z01 MH 00071-0 BP). Normal volunteers are admitted to the unit to provide control data for specific studies in patients and to assess clinical and biological interrelationships in normal as well as patient populations.

2. Behavioral and Psychological Evaluation

a. Psychological Evaluation: Patients are rated twice daily in a double-blind fashion and are assessed with a variety of psychological tests (as previously described). Life Course of Illness is charted graphically in great detail.

b. Biological Evaluation: EEG sleep, AER, and glucose utilization on PET are studied during medication-free intervals, as is procaine activation of EEG, behavior, and cognition. Neurotransmitters, endocrine substances, and peptides are measured in urine, plasma, and CSF before and after acute drug challenges and longer-term treatment. Endocrine tests are described in project #Z01 MH 00452-12 BP.

3. Treatment

Drug evaluation is conducted in a double-blind fashion. Routinely employed drugs include lithium, neuroleptics, tricyclic and MAOI antidepressants. New and experimental treatments include carbamazepine, valproic acid, phenytoin, clonidine, sleep deprivation, and T₃ or T₄ potentiation.

4. Animal Models

A rodent behavioral pharmacology laboratory is maintained in collaboration with Drs. S.R.B. Weiss and A. Pert to develop new research techniques in several areas. The longitudinal evolution of behavioral pathology and its underlying biochemical mechanisms are assessed in different paradigms including: 1) electrophysiological kindling; 2) pharmacological kindling; and 3) behavioral sensitization to psychomotor stimulants such as cocaine. The role of seizures in the development of behavioral pathology is studied utilizing electrical and pharmacological kindling and CRF. The anticonvulsant mechanism of action of carbamazepine is also studied in the kindling paradigm.

C. Major Findings

1. Carbamazepine: A New Treatment and an Alternative or Adjunct to Lithium for Manic-Depressive Illness

a. Acute Antimanic Efficacy: In collaboration with Drs. T. Uhde, K. Kramlinger, and other physicians in the Branch, we have found that carbamazepine is effective in the acute treatment of manic patients, including many who were previously nonresponsive to lithium carbonate. The magnitude and time-course of improvement on carbamazepine paralleled that of neuroleptics. Sleep improved significantly in the first week of treatment. Twelve of 19 (63%) acutely manic patients have shown good responses. These responders, compared to nonresponders, were more severely manic and dysphoric at the onset during their placebo period and they were also more rapid cyclers. Responders had a negative family history for manic-depressive illness in first degree relatives, while nonresponders were equally divided among family history positives and negatives. These data suggest an opposite clinical profile of response to lithium and carbamazepine. While manic severity, dysphoria, rapid-cycling, and negative family history tend to be associated with poorer response to lithium, these variables are associated with better antimanic response to carbamazepine.

Potentialization of carbamazepine with the addition of lithium carbonate resulted in improvement in five of six patients who had previously been inadequately responsive to the antimanic effects of either drug alone.

b. Acute Antidepressant Efficacy: Fifteen of the first 47 patients have shown evidence of a marked clinical response to carbamazepine. Patients with initially more severe depression responded better to carbamazepine than those with less severe ratings of depression. Those with more rapid cycling (episodes/years ill) and hospitalizations for mania, but fewer total weeks depressed (i.e., less chronic depression), also responded better (see also thyroid correlate of response below).

In 15 depressed patients who were inadequately responsive to the acute antidepressant effects of carbamazepine alone administered on a double-blind basis, lithium was added also on a blind basis (with K. Kramlinger). Eight of the 15 patients showed a marked response to this lithium potentiation. The time-course of acute antidepressant response was rapid, and was more rapid than previously observed in responders to lithium treatment alone. These data suggest that lithium potentiates the antidepressant effects of carbamazepine, as has been reported for many other antidepressant modalities, including heterocyclics and monoamine oxidase inhibitors. While the mechanism of action remains to be elucidated, the rapid onset of lithium potentiation has been suggested by de Montigny to involve the serotonin system for more traditional antidepressants. Not only was the time course of acute antidepressant response to lithium potentiation of carbamazepine faster than the antidepressant response achieved with either agent alone, but it was also faster than the onset of antimanic response to lithium potentiation. These data further support the concept that the rapid onset of acute antidepressant effects with lithium potentiation may be achieved by biological mechanisms that are different from those engaged in the antimanic effects or those attributable to either drug alone.

c. Prophylactic Efficacy of Carbamazepine: In a series of lithium-nonresponsive, rapidly cycling manic-depressive patients, the addition of carbamazepine decreased the mean number of both depressive and manic episodes. The severity and duration of episodes and percentage of time ill also tend to be reduced. Potential reasons for loss of efficacy (breakthrough of manic or depressive episodes) during prophylaxis are being studied with G. Leverich.

d. Side Effects of Carbamazepine-Lithium Combination Treatment: The side-effects profiles of carbamazepine and lithium tend to be substantially different, offering the patient the possibility of alternative treatment should one drug or the other not be well tolerated. When the two drugs are used in combination, important interactions are observed. Carbamazepine alone decreases white count in the majority of patients, while lithium increases white count. During lithium potentiation of carbamazepine treatment of 22 depressed or manic patients, Dr. Kramlinger observed that lithium reversed the white count suppression induced by carbamazepine and by the third week of combination treatment, values were actually increased over those observed during the baseline placebo period.

Lithium induces diabetes insipidus while carbamazepine has been used to treat the syndrome. However, because the effects of lithium carbonate occur at a level below the receptor, probably involving adenylate cyclase, carbamazepine is not able to override the effects of lithium and will not reverse lithium-induced diabetes insipidus. While carbamazepine induces mild decreases in serum sodium and calcium, these effects were not significantly reversed by lithium potentiation.

Carbamazepine decreases plasma levels of T_4 , free T_4 , and T_3 without significantly increasing TSH. Lithium potentiation results in further decreases in circulating thyroid hormone levels and increases in TSH secretion. These data suggest that lithium and carbamazepine are impairing thyroid hormone levels at different steps in the regulation of thyroid function. Other data suggest that carbamazepine may increase peripheral thyroid metabolism.

In contrast to lithium carbonate, which can induce clinical hypothyroidism that requires supplemental treatment with thyroid hormone in a small but substantial percentage of patients, hypothyroidism on carbamazepine is rarely induced and has not been observed in our series. In fact, those with the greatest decrements in circulating T_4 and free T_4 have shown the greatest degree of acute antidepressant response to carbamazepine. These paradoxical data are consistent with studies of Baumgartner and associates, indicating that patients with greater decrements in thyroid indices on maprotiline and zimelidine respond better to these treatments.

Rashes have been observed in 13 of 113 patients (11.7%) and carbamazepine was discontinued in each instance. The rashes were uniformly pruritic in nature and, in 12 instances, occurred in the second or third week of carbamazepine treatment.

e. Carbamazepine and its -10,11-Epoxy Metabolite: Levels of carbamazepine itself in plasma or in CSF do not appear to be related to the degree of clinical antidepressant or antimanic response. However, preliminary data suggest that the levels of the active metabolite of carbamazepine, carbamazepine-10,11-epoxide, measured in CSF, were more closely related to the degree of antidepressant response. This metabolite has been demonstrated by us to have anticonvulsant

effects on amygdala-kindled seizures and by others to have antinociceptive properties and to be effective in the treatment of trigeminal neuralgia. Based on these data, we have undertaken a clinical trial of carbamazepine-10,11-epoxide in order to establish whether it has psychotropic properties. The first patient entered in the clinical trial did not respond to the 10,11-epoxide for the treatment of acute mania.

f. Selective Responses to Different Anticonvulsant Agents in Affectively Ill Patients: Response to one anticonvulsant does not appear to produce response to another. We have observed clearcut response to carbamazepine but not to valproic acid or phenytoin in an individual patient completing a double-blind, crossover to these three agents. Conversely, we have observed other patients who are inadequately responsive to carbamazepine who respond to valproic acid. These data are not only of clinical import, but suggest the possibility that differential biochemical or physiological properties of different anticonvulsants may be related to differential clinical responsivities in different patients. This may be particularly apparent in the case of carbamazepine compared to clonazepam, where the two drugs exert differential effects on benzodiazepine receptors, carbamazepine likely interacting with the "peripheral-type" benzodiazepine receptor and clonazepam acting exclusively at the "central-type". We are also beginning to explore the utility of combination treatment in refractory bipolar patients. We have just discharged a patient with a 30-year history of ultra-rapid cycling manic-depressive illness who responded well only after T_3 (50 μ g) had been added to the combination of carbamazepine, lithium, and valproic acid.

The effectiveness of the anticonvulsants carbamazepine, valproate, clonazepam, and related drugs raises the paradox of why both anticonvulsants and the induction of seizures with electroconvulsive therapy (ECT) are useful treatments for acute manic and depressive illness. We have observed that electroconvulsive seizures in the rat are paradoxically anticonvulsant to amygdala-kindled seizures. These data raise the possibility that common biochemical and physiological mechanisms of electroconvulsive therapy and anticonvulsants such as carbamazepine could be related to their profile of therapeutic efficacy in both phases of affective illness.

g. Studies of Carbamazepine's Mechanism of Action:

1) Effects on Classical Neurotransmitters and Modulators:

Evidence of others in laboratory animals suggests that carbamazepine blocks the reuptake of norepinephrine (NE), but also inhibits stimulated-induced release; it increases firing of the locus coeruleus, but decreases NE turnover. Elevated levels of CSF NE in mania are decreased by carbamazepine.

New evidence from our laboratory suggest that noradrenergic effects of carbamazepine are important to its anticonvulsant properties. An alpha-2 mechanism is likely involved in the anticonvulsant effects, as the alpha-2 antagonist, yohimbine, reverses the effects of carbamazepine on amygdala kindling. Alpha-2 effects may be necessary but not sufficient for the anticonvulsant effects, as the alpha-2 agonist clonidine is not an effective anticonvulsant on this model of kindled seizures.

Although it is as effective as the neuroleptics in the treatment of acute mania, carbamazepine does not block dopamine receptors or produce other typical neuroleptic effects. Moreover, it has not been associated with the development of

parkinsonian side effects or with the syndrome of tardive dyskinesia as have the neuroleptic drugs. These data suggest that carbamazepine acts by mechanisms other than blockade of dopamine receptors.

Alterations in GABA have been postulated in affective illness (see below) as well as in the seizure disorders. Carbamazepine has been reported to decrease the turnover of GABA in animal studies (Bernasconi, 1984), although brain levels are not altered by the drug. This is consistent with our data indicating that CSF GABA levels are not significantly decreased during treatment with carbamazepine compared to baseline levels.

GABA-B (baclofen type) mechanisms are implicated in the antinociceptive actions of carbamazepine based on animal models of trigeminal neuralgia. For example, Terrence et al (1983) reported that an inactive isomer, d-baclofen, reversed the antinociceptive effects of carbamazepine and the active isomer l-baclofen. In contrast, we have demonstrated that the anticonvulsant effects of carbamazepine on amygdala kindling do not appear to involve GABA-B mechanisms, as these effects are not reversed by d-baclofen.

Thus, it remains to be determined whether the effects of carbamazepine in manic-depressive illness are more akin to those in trigeminal neuralgia (potentially involving GABA-B mechanisms) or in seizures (such as amygdala kindling that do not involve GABA-B mechanisms). In order to assess these differential possibilities, we have undertaken a clinical trial of the active isomer, l-baclofen, in the treatment of manic-depressive patients. The first two patients have been entered in the clinical trial and efficacy has not been demonstrated in these two patients treated with doses up to 10 mg/day (the initial limit imposed by the FDA). Further patients will be tested at higher doses when FDA approval is obtained. Most effective antidepressants have been reported to increase GABA-B receptors in frontal cortex (Lloyd et al, 1987), giving further rationale to the baclofen trial.

Effects of carbamazepine on central and "peripheral-type" benzodiazepine receptors have been studied with biochemical techniques (Marangos et al.), and electrophysiologically in the amygdala kindling model (Weiss et al). Carbamazepine binds poorly to the central site (^3H -diazepam or ^3H -BCCe), but more potently at the Ro5-4864 (peripheral) site. Dr. S.R.B. Weiss has found that Ro-15-1788 and BCCM block the anticonvulsant actions of diazepam, but are ineffective in reversing the anticonvulsant effects of carbamazepine on amygdala kindling. Conversely, Ro5-4864 does reverse the anticonvulsant effects of carbamazepine and its 10,11-epoxide, but not those of diazepam. PK-11195, which acts selectively at the peripheral site, blocks the effects of Ro5-4864 on carbamazepine's anticonvulsant effects. Taken together, these biochemical and electrophysiological data suggest that carbamazepine may exert its anticonvulsant effects through the "peripheral-type" but not the "central-type" benzodiazepine receptor, and thus act very differently from diazepam and clonazepam. It is of interest that "central-type" benzodiazepine receptors are linked to chloride channels, while the "peripheral-type" may be linked to calcium channels.

Carbamazepine is potent in displacing binding of adenosine receptor ligands (Marangos et al.). Contrary to predictions, chronic treatment with carbamazepine (similar to that with caffeine) increased the number of adenosine receptors, sug-

gesting that carbamazepine may possess adenosine antagonist-like properties. It does not appear that carbamazepine exerts its anticonvulsant effects through the adenosine receptor, as its effects on kindled seizures are not altered by several adenosine-active agents (S.R.B. Weiss). The increase in adenosine receptors following carbamazepine treatment was long lasting (possibly permanent), suggesting a novel mechanism for this effect.

2. Carbamazepine's Effects on Endocrine and Peptide Systems

Carbamazepine significantly decreased somatostatin measured in CSF of affectively ill patients (studied in collaboration with Drs. D.R. Rubinow, P.W. Gold, and S. Reichlin). These findings, which have recently been replicated in neurological patients (Steardo et al, 1986) are of interest in relationship to the reports by others of long-lasting increases in brain somatostatin following amygdala kindling seizures and observations that depletions of somatostatin exert anticonvulsant effects on kindled and CRF seizures. Thus, changes in somatostatin could relate to the anticonvulsant properties of carbamazepine.

Carbamazepine may directly or indirectly potentiate vasopressin effects at the receptor level. Rubinow and associates have found that carbamazepine induces escape from dexamethasone suppression and increases urinary free cortisol excretion. Carbamazepine did not alter CSF opiate binding activity in affectively ill patients, or affect morphine's antinociceptive effects on tail flick latencies in the rat. In contrast to lithium, carbamazepine inhibits rather than potentiates the TSH response to TRH.

3. Physiological and Behavioral-Pharmacological Effects of Carbamazepine

The anticonvulsant effects of carbamazepine have been examined in several different types of kindling. Remarkable dissociations in the anticonvulsant efficacy of carbamazepine have been revealed as a function of stage and type of kindling. For example, carbamazepine is ineffective in blocking the development of electrical kindling of the amygdala in the rat, even though it is highly potent in blocking completed amygdala-kindled seizures. Conversely, carbamazepine blocks the development of lidocaine- and cocaine-kindled seizures, but acutely is ineffective against completed or high-dose local anesthetic seizures. Pinel (1983) has demonstrated that spontaneous seizures which occur after many hundreds of kindled seizures are also differentially pharmaco-responsive compared with the early stages of kindling. Thus, there appears to be a general principle that different stages in the evolution of kindling -- developmental, completed, and spontaneous -- may be differentially responsive and, therefore, involve different neuroanatomical, physiological, and/or biochemical mechanisms. Different types of kindling are also differentially responsive.

The bulk of our work has utilized completed kindled seizures in order to elucidate possible mechanisms of anticonvulsant effects of carbamazepine. Our data strongly implicate noradrenergic alpha-2 mechanisms and "peripheral-type" benzodiazepine receptor mechanisms in this effect. Data from other investigators also suggest that stabilization of type-2 sodium channels are likely involved in the anticonvulsant effects of both carbamazepine and phenytoin.

However, our time course analysis of the clinical efficacy of carbamazepine in epilepsy, pain syndromes, and affective illness suggests that the mechanisms under-

lying the anticonvulsant effects of carbamazepine, which occur almost immediately, may be different from those underlying efficacy in mania and depression, which require some 2-3 weeks before they become fully manifest. Based on this analysis, one would want to investigate anticonvulsant mechanisms that require time to develop in order to have a more parallel model for the time frame of efficacy occurring in manic-depressive illness. The effects of carbamazepine on cocaine-induced seizures described below appear to be a useful paradigm in this regard.

In contrast to electrical kindling of the amygdala, which is poorly responsive to carbamazepine in the early developmental stage, the development of pharmacological kindling with local anesthetics is robustly inhibited by carbamazepine. Carbamazepine almost completely blocks the development of lidocaine-kindled seizures and also is highly effective in blocking the development of cocaine-kindled seizures in three separate studies at doses of 40, 50, and 65 mg/kg, respectively. At these doses, animals begin to rapidly develop seizures to a dose of drug that was previously non-convulsive and, in contrast to lidocaine seizures which are well tolerated, those associated with cocaine are extremely lethal. Carbamazepine not only blocks the development of cocaine-kindled seizures, but markedly reduces its associated lethality. In spite of this robust effect on the development of local-anesthetic-induced kindling, carbamazepine is ineffective in blocking completed lidocaine-kindled seizures or high-dose cocaine seizures following acute administration.

Thus, an interesting dissociation occurs; completed amygdala-kindled seizures are responsive to the acute anticonvulsant effects of carbamazepine, while local-anesthetic seizures are unresponsive to this manipulation. However, if carbamazepine is administered chronically prior to the local-anesthetic seizures, anticonvulsant efficacy is observed. Therefore, dissection of the anticonvulsant mechanisms which require chronic administration of carbamazepine in order to become manifest against cocaine-induced seizures might provide a suitable first-line strategy for approximating mechanisms which might also be involved in the psychotropic effects of carbamazepine, which also require chronic administration before they are observed.

Lithium carbonate is ineffective in blocking either the development of amygdala-kindled seizures or completed kindled seizures, yet appears to have some efficacy in blocking cocaine-induced behavioral sensitization (described below). Conversely, carbamazepine is a highly effective anticonvulsant for some types of kindled seizures, but is without effect in blocking cocaine-induced behavioral sensitization. Thus, comparison and contrast of the effects of lithium and carbamazepine not only on a biochemical but also on a physiological and behavioral/pharmacological basis may help to elucidate different mechanisms of action of these two compounds, which are both effective in manic-depressive patients.

The amygdala-kindling data suggest that different stages in the development of epilepsy will also show differences in anticonvulsant responsiveness. One might ask whether a similar principle exists for treatment of different stages in the evolution of affective illness. A considerable body of data suggest that lithium carbonate is less effective in the rapid or continuous cycling type of illness, which often occurs late in the course of affective illness, while these patients may be among those who respond best to carbamazepine. Thus, in addition to a variety of other characteristics that may differentiate lithium responders from carbamazepine

responders, work with the animal models suggests the utility of considering stages in the developmental longitudinal course of the illness as a relevant variable, with the possibility emerging that carbamazepine and related anticonvulsants may be most useful in the later stages of affective illness, which tend to be less responsive to treatment with lithium carbonate.

2. Approaches to Classical Neurotransmitter and Peptide Dysfunction in Affective Illness

a. CSF norepinephrine (NE) is significantly increased in manic patients compared to either of the other patient or control populations. Indirect biochemical, pharmacological, and endocrine data continue to suggest a role for dopamine in some aspects of affective illness. Dopamine and its metabolite HVA and DOPAC are studied, in collaboration with Drs. D. Rubinow and M. Linnoila, in the CSF of depressed, manic, and euthymic patients and controls. Studies of the relationship of plasma HVA to the longitudinal course of affective illness suggest only weak relationships to mood or anxiety in selected individuals, but no consistent relationship like those reported for plasma HVA and severity of psychosis in schizophrenics.

b. Dr. Rubinow found that CSF somatostatin is significantly decreased in depressed patients compared to those re-studied in the euthymic state or compared to normal volunteer controls. There have now been seven replications in other laboratories of the finding of low somatostatin in depressives compared to normals or other psychiatric comparison groups. These findings are of interest in relationship to the reports of decreased somatostatin in brain and CSF of patients with Alzheimer's disease and several other neuropsychiatric syndromes that can present with cognitive defects, including multiple sclerosis and parkinsonism. Dr. Rubinow, in collaboration with Drs. A. Doran, D. Pickar, A. Roy, and S. Paul, has also found that depressed and schizophrenic patients who were cortisol hypersecretors, as indicated by escape from dexamethasone suppression, had significantly lower CSF somatostatin. The causal links in the relationship are unclear, as somatostatin and cortisol can each influence the other; for example, glucocorticoid treatment of normal volunteers decreases CSF somatostatin (Wolkowitz & Rubinow).

Dr. Gold has completed a series of studies of CRH infusions in affectively ill patients and controls (as described in Project # Z01 MH 00452-12 BP) and found evidence for blunted ACTH response in depressive but not manic or improved states. In contrast to depressed patients, hypercortisolemic patients with Cushing's disease show ACTH hypersecretion to CRF, providing a possible differential diagnostic test.

3. Course of Affective Illness: Relationship to Biochemical and Clinical Variables

As described in detail in last year's annual report, we have characterized the course of more than 100 affectively ill patients' on our clinical research unit over the past several years. These descriptive data are of considerable interest in their own right, but also form a critical substrate for analyzing the relationship of entire course of illness to subsequent pharmacological response (see data on carbamazepine above) as well as to various neurobiological alterations observed in the illness. An example of this usefulness is derived from our recent study of the relationship of thyroid dysfunctions in manic-depressive illness. It

had previously been reported by others that patients with hypothyroidism were overly represented in a group with rapid cycling.

a. Thyroid Function and Course of Illness: In order to more systematically examine this question, we assessed thyroid indices at several points during the NIMH hospitalization in relationship to retrospective and prospective course-of-illness variables. Values were obtained at the onset and termination of the medication-free observation interval at NIMH and during treatment with both lithium and carbamazepine. Thyroid values changed from the first to the second medication-free period in a highly consistent fashion. T_4 and free T_4 levels increased while T_3 levels decreased, suggesting decreased conversion of T_4 to T_3 consistent with the development of a euthyroid sick syndrome. Depression levels did not change from the first to the second interval although there was a small increase in psychosis rating indicating that the patients remained ill during this period of time and did not show spontaneous remissions. The changes in the thyroid indices were most pronounced in the patients who had been rapid cyclers (demonstrating more than four episodes per year in the year prior to NIMH admission) and in those who demonstrated hypercortisolism (increased excretion of urinary free cortisol) during their medication-free evaluation at NIMH. These data are of interest in relationship to reports that a variety of medical illnesses and glucocorticoid treatment can induce the euthyroid sick syndrome.

We observed a pattern of increased thyroid indices being positively correlated with measures reflecting increased rapidity of cycling and a greater severity of illness. Moreover, rapid cyclers had significantly higher levels of T_4 and free T_4 than non-rapid cycling patients. There was no relationship of TSH levels to degree of rapid cycling. Duration of time off lithium did not account for the findings and was unrelated to differences in thyroid indices. In fact, in a subgroup of patients exposed to lithium, the relationship of rapid cycling to higher levels of thyroid hormone remained.

Thus, a consistent perspective on the relationship of thyroid dysfunction to affective illness emerges in our data. Rapid cyclers are characterized by relative hyperthyroid, rather than hypothyroid, indices (although most values are within the normal range) and respond to drugs that tend to further reduce thyroid indices, such as lithium and carbamazepine (see above). Moreover, we observed that responders compared to nonresponders to the acute antimanic effects of carbamazepine showed significantly greater levels of T_4 and free T_4 in the period prior to carbamazepine treatment. These data are also consistent with the findings of others that some 30-40% of affectively ill patients show blunted TSH responses to TRH and only a small minority show increased responses. These data from the literature are also consistent with relative thyroid hyperfunction rather than hypofunction. Moreover, recent data from Nemeroff et al indicate increased TRH levels in the CSF of depressed patients, and Reichlin and associates reported that chronic intrathecal TRH administration for patients with amyotrophic lateral sclerosis produces a profile of thyroid indices similar to that observed in depressed patients with normal to high hormone levels and a blunted response to TRH.

Joffe et al have suggested that this view of relative thyroid hyperfunction in affective illness may also be consistent with the data that antidepressant responses can be potentiated with supplemental thyroid hormone administration with T_3 .

T_2 , by feedback inhibition, actually suppresses circulating levels of T_4 . Since CNS uptake is dependent on circulating levels of T_4 and intracellular conversion to T_3 , this thyroid manipulation may actually induce relative thyroid hypofunction, like many of the other treatments of affective illness. Joffe would predict, on the basis of this hypothesis, that depressed patients would respond better to T_3 potentiation than to T_4 , and preliminary data from his group in Toronto now support this prediction.

b. Suicidality and Course in Affective Illness: We have found that 49 of 87 of our affectively ill patients (56%) had made suicide attempts. Females (34/51 or 66%) were more likely than males (15/36 or 41%) to have made an attempt. While the majority of attempts occur within the first year or two of illness, more severe attempts (as assessed by a formal "risk" scale) were significantly correlated with duration of illness ($r = .52, p < .004$, age corrected) and total number of affective episodes ($r = .40, p < .03$). Intensity of suicidal ideation (which was higher in attempters than non-attempters) was not highly correlated with lethality of attempt, but was correlated with several variables including number of episodes of illness. This study provides one of the first attempts to examine suicidality in affectively ill patients as it relates to the longitudinal course of affective illness.

4. Depressive Subtypes and Symptoms in Relation to Regional Localization of Function

a. Psychosensory Phenomena: In collaboration with Dr. P. Hauser, we have continued to assess signs and symptoms that are usually associated with psychomotor epilepsy in patients with primary affective illness and panic-anxiety illness, as well as in patients with temporal lobe epilepsy and in a medical control group of hypertensive patients. Compared to the medical control group, patients with affective illness, panic-anxiety disorders, and with epilepsy showed a highly significant increased incidence in the number of these signs and symptoms. The qualitative symptom profiles differ slightly among the affective, anxious, and epileptic patients. Depressed patients with a history of panic attacks have more symptoms than depressed or anxious patients without panic attacks and show a profile highly characteristic of panic patients. To the extent that psychosensory distortions and related symptoms usually associated with temporal lobe epilepsy are occurring with a high incidence in patients with primary affective illness, these data might suggest that some of the neural substrates involved in complex partial seizures overlap with affective illness. Contrary to predictions, affective patients with greater numbers of psychosensory symptoms responded better to lithium carbonate, and preliminary data suggest that this is not the case for carbamazepine.

b. Psychological, Structural, Metabolic, and Electrophysiological Approaches to Regional Brain Function in Affective Illness: A variety of psychological test batteries are employed to assess possible alterations in regional brain function in patients with affective illness, including the Luria Battery and the Halstead Categories Test. Impairment in cognitive function has been documented on these tests during depression. Depressed patients compared to controls are also deficient in their ability to recognize emotions in pictures of faces presented to them; recognition of expressions of sad and elated are particularly disturbed (Rubinow et al).

Computerized axial tomography (CAT) scans have been performed on our patients with affective illness and reveal a similar range of ventricular brain ratios (VBRs) comparable to those observed in schizophrenic patients. Larger VBR is not associated with a more chronic or recurrent course of illness in manic-depressive patients, as has been reported in some studies of schizophrenics. Affectively ill patients also do not show gross abnormalities of brain structure assessed by magnetic resonance imaging (MRI), as studied by Dr. P. Hauser.

Positron emission tomography (PET) scan studies using 2-deoxyglucose indicate glucose utilization in temporal cortex relative to other areas in the same brain slice was also lower in depressed patients compared to controls (studied with Drs. Cohen, DeLisi and Buchsbaum). These data provide evidence that depressed patients differ from patients with complex partial seizures who show areas of increased glucose utilization in the temporal lobe ictally and hypometabolism interictally (Engel et al, 1982).

c. Procaine Infusions as a Probe of Limbic System Responsivity:

Graded doses of the local anesthetic procaine were administered to affectively ill patients (in collaboration with Drs. C. Kellner, F. Putnam and M. Kling), borderline personality disorders (in collaboration with R. Cowdry and D. Gardner), and normal volunteers in an attempt to probe limbic system responsivity. Analysis of the first 21 subjects by Dr. R. Coppola reveals selective increases in fast EEG activity, especially 26 to 45 Hz over the temporal cortex, confirming in man the suggestions from animal studies that local anesthetics activate temporal lobe and limbic structures. Dose-related alterations in subjective sensory and cognitive functions were reported as well as a variety of affective responses ranging from mood elevation to dysphoria. Vivid recall of experientially immediate memories, as well as hallucinatory-like phenomena, occurred less often. In patients with borderline personality disorder, degree of fast activation of the temporal cortex was not positively correlated with response to carbamazepine (Cowdry and Gardner). Procaine-induced release of ACTH, cortisol, and prolactin, but not growth hormone, has also been documented in collaboration with Drs. P. Gold, C. Kellner, and M. Kling. These data suggest the utility of procaine as a potential pharmacological probe of the limbic-temporal lobe function.

5. Laboratory Studies of Behavioral Sensitization and Electrophysiological Kindling (in collaboration with Drs. S.R.B. Weiss and Agu Pert)

a. Conditioning in Cocaine-induced Behavioral Sensitization: We have investigated the phenomenology and mechanisms underlying the increased behavioral responsivity to the same dose of the psychomotor stimulant cocaine. Animals administered cocaine (10 mg/kg i.p.) once-daily show increasing amounts of locomotor hyperactivity and stereotypy to the same dose over time. An environmental context or conditioning component has been demonstrated. For example, animals repeatedly treated with cocaine in the same context (the test cage) showed greater degrees of hyperactivity and stereotypy than animals receiving identical doses in a different environment and then injected in the test cage. These findings have been replicated using drug or saline injections into the nucleus accumbens; cocaine pretreated animals showed an increased response to intracerebral saline or amphetamine only when they had been pretreated in the same environment. The similarity of the pretreatment environment (where and in what type of cage animals receive cocaine [40 mg/kg]

on day 1) to the test environment where they receive cocaine (10 mg/kg) on day 2 is also related to the degree of sensitization.

The experience of motor hyperactivity itself in the pretreatment environment following cocaine challenge (40 mg/kg) appears necessary for cocaine-induced behavioral sensitization to occur to a cocaine (10 mg/kg) test dose the next day. If cocaine-induced activity during the pretreatment is blocked with haloperidol, diazepam, but not muscimol, sensitization to cocaine (10 mg/kg) does not occur. (Diazepam and muscimol pretreatments in themselves increase subsequent responsivity to cocaine, suggesting that GABA mechanisms may facilitate subsequent response to cocaine.)

b. Neuroleptics Block the Development, But Not Expression of Cocaine-induced Behavioral Sensitization -- A Model of Neuroleptic Nonresponsiveness: It is also of interest that while neuroleptic blockade of cocaine-induced hyperactivity during the day 1 cocaine pretreatment phase blocks sensitization, neuroleptic administration prior to the day 2 testing phase does not block the sensitizing effect of cocaine. Two doses of haloperidol (0.2 mg/kg and 0.5 mg/kg) that were sufficient to block the development of sensitization when administered on day 1, were both unable to block the expression of sensitization when administered on day 2. To the extent that behavioral sensitization accounts for some of the progressive development of psychopathology to cocaine in man, these data suggest that neuroleptic treatment, once sensitization has already developed, will be ineffective. These findings may also represent an animal model for neuroleptic nonresponsiveness in some psychotic conditions.

c. Anatomical Substrates for Cocaine-induced Behavioral Sensitization: Using lesion strategies, we have attempted to dissect possible neural substrates mediating the conditioned component of cocaine-induced behavioral sensitization. We found that selective dopaminergic lesions of the nucleus accumbens that were insufficient to block day 1 high-dose cocaine-induced hyperactivity did block the expression of cocaine-induced behavioral sensitization. Similarly, electrolytic lesions of the amygdala as well as selective dopaminergic lesions of the amygdala, blocked cocaine-induced behavioral sensitization. This effect was not achieved by lesions of the dorsal or ventral hippocampus or midline cerebellar structures. These data suggest that nucleus accumbens and amygdala and, in particular, the dopaminergic components of these pathways, may be involved in the mediation of cocaine-induced behavioral sensitization.

d. Pharmacological Kindling: Repeated, intermittent electrical stimulation of the brain results in increasing duration, spread, and complexity of electrical after-discharges culminating in the appearance of major motor seizures to a previously subthreshold stimulation (Goddard et al, 1969). We have employed this procedure in order to study long-lasting changes in neural and behavioral excitability that accompany this process. Repeated daily injections of the same dose of lidocaine (65 mg/kg, i.p.) also lead to an increasing incidence, severity, and duration of seizures to the same dose over time, a phenomenon we have called pharmacological kindling. Cocaine (65 mg/kg) also produces pharmacological kindling, but with a much higher seizure incidence and lethality.

Carbamazepine is a potent inhibitor of the development phase of lidocaine-kindled seizures, but is ineffective against completed lidocaine seizures.

Carbamazepine also slows the development of cocaine-kindled seizures and their accompanying lethality, but is ineffective in preventing high-dose cocaine-induced seizures and may even increase the cocaine-induced lethality (S.R.B. Weiss). However, chronic carbamazepine does decrease cocaine-induced seizures and lethality.

e. CRF Seizures and Behavior: Interaction with Amygdala Kindling:

Dr. S.R.B. Weiss, in collaboration with Dr. A. Pert, has conducted a series of studies on the behavioral and convulsive effects of corticotropin releasing hormone (CRF) administered intracerebroventricularly. CRF induces the late onset (i.e., following a lag of approximately 4-8 hours post injection) of seizures that behaviorally and electrophysiologically resemble those produced from electrical stimulation of the amygdala. Following five repeated once-daily administrations, tolerance develops to the seizure inducing effects of CRF. Despite this, CRF seizures enhanced the development of amygdala-kindled seizures such that animals pretreated with CRF develop electrically kindled seizures twice as fast as vehicle-injected controls. CRF-treated animals also show increases in aggressive behavior toward other rats, an effect that was markedly enhanced in the electrically kindled rats. Conversely, electrically kindled rats showed a decreased convulsive response to CRF similar to that seen with repeated CRF injections.

The convulsive response to CRF was not reliably reproduced by local intracerebral injection into amygdala, hippocampus, septum, hypothalamus, periaqueductal gray (PAG) or the pre-pyriform area identified by K. Gale as a highly sensitive trigger zone for other seizures. However, the aggressive behavior could be elicited by CRF injections into PAG. Moreover, small lesions of the amygdala decreased the CRF-induced aggression following i.c.v. administration, but small or large amygdala lesions did not affect the development of seizures. Lesions of the hippocampus, pre-pyriform area, and olfactory tubercle similarly did not block the development of seizures produced by i.c.v. CRF.

These data suggest that CRF is inducing seizures behaviorally similar to those produced by electrical stimulation of the amygdala, but they are not dependent on an amygdala substrate for their occurrence. Further, these data suggest the possibility that an endogenously produced, stress-related peptide such as CRF may, under pathological conditions, be associated with alterations in convulsive and aggressive responsivity. No discrete brain focus of this effect has so far been found and since 50-100 µg of CRF into the CSF appears to be required, this effect may be pharmacological rather than physiological.

D. Proposed Course of Project

We have helped to introduce and document carbamazepine as an effective treatment modality for manic-depressive and schizoaffective illness. Preliminary predictors of clinical response have been elucidated. We propose to further delineate clinical and biological markers of carbamazepine response. Preliminary evidence suggests that many patients who clearly do not respond to lithium carbonate will respond to carbamazepine. It will be increasingly important to establish whether response to carbamazepine, compared to lithium carbonate, delineates separate subgroups of patients with affective illness. As such, carbamazepine responders might be distinguished on the basis of: 1) severity; 2) pattern (rapid

cycling); 3) genetics (family history negative); 4) course of illness (late); or 5) biological markers.

The degree of generalization of carbamazepine response to other anticonvulsant agents such as phenytoin or valproic acid will be another area of both clinical and theoretical import. This is also particularly the case in light of our recent findings that electroconvulsive shock exerts potent anticonvulsant effects on limbic system seizures. Are anticonvulsant effects of a variety of treatment modalities (including ECT) linked to therapeutic response in affective illness? Carbamazepine is clearly useful in pain syndromes that do not involve a convulsive process, and effectiveness of anticonvulsant agents in a subgroup of patients with affective illness does not imply an underlying ictal process.

The possible mechanisms of action of carbamazepine in our patients, as well as in behavioral pharmacological models, will also be pursued. A clinical trial of baclofen will help elucidate the role of GABA-B mechanisms in carbamazepine's efficacy. We will investigate whether carbamazepine's anticonvulsant metabolite, carbamazepine-10,11-epoxide, also has important psychotropic properties in manic-depressive patients.

Alterations in somatostatin as they relate to affective and seizure mechanisms will also be systematically explored, especially in light of growing evidence of alterations in somatostatin in depression and in a variety of neuropsychiatric disorders (D.R. Rubinow).

As described in detail in Project #Z01 MH 00071-07 BP, Dr. T.W. Uhde will continue to explore the similarities and differences in patients with panic anxiety syndromes and those with affective illness in terms of acute symptomatology, longitudinal course of illness, and response to pharmacological agents. Catecholamines appear to be altered in both the mood disorders and in panic anxiety disorders. Response to treatments which act on catecholamine systems such as clonidine will be compared and contrasted in both patient populations. Since caffeine has been shown to increase plasma cortisol and induce escape from dexamethasone suppression, the clinical, mechanistic, and theoretical implications of this important observation will be systematically followed up by Dr. Uhde and his associates.

Dr. D.R. Rubinow is continuing to study and treat patients with menstrually-related exacerbation of mood and behavior disorders. He will be examining this problem from a clinical and endocrinological point of view, and as a model for studying the acute onset and offset of affective dysfunction.

Work in animal models will continue to focus on possible mechanisms underlying behavioral sensitization and electrophysiological kindling. In collaboration with Drs. S.R.B. Weiss, P. Marangos, and J. Patel, neurotransmitter receptors, protein phosphorylation, and ion channels will be examined as possible mediators or modulators of the electrophysiological kindling paradigm. The mechanisms of anticonvulsant action of carbamazepine on amygdala-kindled seizures will also be further studied. The role of environmental context and conditioning will also be examined in these paradigms; anatomical and biochemical substrates will be studied. The mechanisms of cocaine-induced kindled seizure lethality will be explored.

E. Significance to Biomedical Research and the Program of the Institute

Based in part on work in this Branch, carbamazepine has emerged as a new treatment for manic-depressive illness. The anticonvulsants have emerged as a class of new treatments as valproic acid and clonazepam also appear effective in mania. Thus, a whole new range of treatment options has evolved from the work with carbamazepine.

Carbamazepine's clinical and theoretical importance is further highlighted by the fact that it is effective in some patients who do not respond to lithium carbonate. Studies of the mechanism of action of carbamazepine may provide new leads to the understanding of mechanisms of action of other effective antimanic and antidepressant drugs as well as basic mechanisms underlying affective dysregulation. Mechanisms can now be compared and contrasted with lithium and also with a range of other anticonvulsants that are effective in manic-depressive illness. Thus, basic and clinical research has led to important findings in neurobiology and the development of a new treatment for affective illness with carbamazepine.

Study of endocrine and peptide substances in man and animals may also provide new conceptual and practical treatment approaches to the relationship between manic and depressive symptoms and biochemistry. Examination of the interaction between classical neurotransmitters and the peptides should prove fruitful in understanding normal and pathological functioning. The multi-disciplinary assessment of our patients' mood, behavior, cognition, physiology, and biochemistry should allow more precise characterization of important biobehavioral relationships and their underlying neural substrates.

Elucidating the mechanisms underlying behavioral sensitization and kindling, which appear to involve processes akin to memory, may provide important information regarding the coding of behaviorally relevant long-term changes in the CNS. Kindling and behavioral sensitization have aided in the conceptualization of a variety of psychiatric disorders that show progressive increases in behavioral pathology over time, and have led to the introduction of new treatment strategies as well as the novel conceptualization of the efficacy of pharmacotherapy as a function of state of syndrome development. This is clearly documented with neuroleptics, which block the development, but not expression, of cocaine-induced behavioral sensitization, and with carbamazepine, which blocks completed amygdala-kindled seizures, but not their development.

Studies of chronic cocaine indicate a potent conditioned component to behavioral sensitization. Conditioning is now also being recognized as a major factor in the clinical treatment of cocaine-addicted patients. If subjects are exposed to cocaine-related paraphernalia or similar cues even months after they have been withdrawn from the drug, powerful craving and/or withdrawal symptoms can be re-introduced. Thus, elucidation of the mechanisms and anatomical and biochemical pathways involved in conditioned components of cocaine-induced behavioral sensitization in the preclinical animal models should lead to a better understanding of related phenomena and possible new treatment alternatives in man.

Similarly, understanding the mechanisms involved in the high lethality of cocaine-induced kindled seizures may lead to better treatment interventions for this potentially catastrophic reaction. Both the behavioral sensitization and

kindling perspective, to the extent that they can be extrapolated to the human cocaine use condition (and all of the data so far suggest that they can be), imply that there may be additional hidden liabilities to cocaine use beyond those that are widely known. That is, that both behavioral and convulsant toxicity may become an increasing problem with repeated use and that a given dose of cocaine, which was previously well tolerated, upon sufficient repetition, may not only lead to increasing pathological effects on behavior, but, in some instances, may produce lethal seizures as observed in pharmacological kindling. Thus, these preclinical findings which are of interest in their own right and as potential models for manic and other psychotic symptomatology and psychiatric disorders, may also be of considerable public health interest and pave the way for reconceptualization of the pathophysiology of cocaine-related syndromes and new treatment interventions.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00071-07 BP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychobiological Correlates and Treatment of Panic and Related Mood Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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LAB/BRANCH

Biological Psychiatry Branch

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TOTAL MAN-YEARS:

5

PROFESSIONAL:

4

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patients with pathological degrees of anxiety who meet DSM III-R criteria for panic disorder, with or without agoraphobia; generalized anxiety disorder; or social phobia are evaluated using psychological, physiological, and biochemical methodologies. Patients with major affective illness, particularly those with a significant anxiety component, are also eligible for participation in the program. Particular attention is given to the role of the noradrenergic, dopaminergic, adrenergic, and serotonergic neurotransmitter systems as assessed by: 1) measurement of the metabolites MHPG and HVA in plasma; 2) adrenergic receptor number and function in platelets; and 3) neuroendocrine and behavioral responses to the alpha-2 adrenergic agonist clonidine and antagonist yohimbine and the serotonin agonist m-chlorophenylpiperazine (MCPP). Research investigating the relationship of noradrenergic and adenosinergic function to other neurotransmitter systems and the hypothalamic-pituitary-adrenal axis also has been initiated. Caffeine and nifedipine challenges are administered to assess their behavioral and biochemical effects. Other approaches to understanding the pathophysiology of anxiety and its potential treatment with alprazolam, carbamazepine, clonidine, imipramine and verapamil will be explored.

An animal model using genetically "nervous" and "normal" pointer dogs has been developed and studied in relation to noradrenergic and adenosinergic function.

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I. Project Description

A. Objectives

This project employs a multidisciplinary team in the study and treatment of pathological anxiety, major affective and related mood disorders.

B. Methods Employed

1. Subjects

a. Patients who meet Research Diagnostic Criteria for panic, phobic, and generalized anxiety disorders, as well as patients who meet DSM III criteria for major affective illness, are candidates for participation in the project. Inpatients are studied and treated on the 3-West Clinical Research Unit and outpatients are followed through the Ambulatory Care Research Facility. A number of previously validated scales to measure state and trait anxiety are utilized and an analogue anxiety scale and panic anxiety scale have been developed to more adequately assess the relationship among state anxiety, phobic anxiety, avoidance behavior, and depressive symptomatology.

b. Normal volunteers are also accepted into the project to provide control data, as well as to assess the relationship between normal state anxiety and selected psychological and biological variables.

2. Psychological and Biological Evaluation

a. Baseline Evaluation. During an initial evaluative period patients undergo extensive neurological, psychological, biochemical, and neurophysiological evaluation. This initial evaluation is indicated due to the heterogeneous nature of the panic and phobic disorders. Anecdotal reports suggest that many medical illnesses may present as or exacerbate pre-existing conditions of pathological anxiety.

b. Life Chart Methodology. A life chart technique has been developed to plot the frequency, intensity, and interval between panic attacks. The character and the change in the quality of panic attacks is assessed as a function of duration and longitudinal course of illness. This approach allows the Unit to document the development, recurrence, and progression of the panic and phobic disorders. Life charting is an important aspect of the overall project because few systematic studies have been conducted on the natural progression of these disorders.

As part of this assessment, life events and their impact on the course of illness are investigated with the PERI-M life events inventory. Moreover, the influence of personality (DSM III, Axis II diagnosis) on the phenomenology and course of illness is systematically evaluated with the Structured Interview for DSM III Personality Disorders (SIDP). These studies are conducted in collaboration with Drs. K. Kramlinger, T. Mellman, P. Roy-Byrne, and with M. Geraci, G. Leverich, and B. Scupi.

c. Sleep and Sleep Deprivation. Electroencephalographic sleep recordings are obtained for three consecutive nights. Although many panic anxious patients, like endogenously depressed individuals, have improved sleep following treatment with tricyclic and monoamine oxidase inhibitors, little is known about the sleep architecture of panic and phobic anxious patients. The effects of one night's sleep deprivation on mood and behavior are investigated in patients with panic disorder and major depressive disorder. Sleep studies are conducted in collaboration with Drs. T. Mellman and P. Roy-Byrne.

d. Galvanic Skin Response. The effects of yohimbine on physiological measures of galvanic skin response, reaction time to auditory tones, pulse, and respiratory rate are studied in panic and phobic anxious patients and age-matched normal volunteers. This investigation is performed in collaboration with Drs. M. Albus, B. Vittone, and T. Zahn.

e. Computerized Axial Tomography. Cerebral CAT Scans are obtained, and, in collaboration with Dr. C. Kellner, cerebral ventricular size is determined in patients with panic disorder. Scans are performed on a GE 8800 or 9800 Scanner.

f. Psychomotor Activity. Twenty-four hour motor activity is assessed with a miniaturized activity monitor worn on the wrist of patients with primary anxiety disorders under a variety of experimental conditions.

g. Caffeine. Caffeine is administered to panic patients and normal controls to assess behavioral and biochemical responses to this agent whose effects are thought to be mediated through the adenosine, GABA-benzodiazepine, and noradrenergic systems.

To assess the effects of caffeine on plasma adenosine, an HPLC assay for adenosine and caffeine has been developed in collaboration with Drs. N. Salem and P. Marangos. The clinical studies are conducted in collaboration with Drs. L. Bierer, J.-P. Boulenger, T. Mellman, R. Post, and M. Geraci and S. Sinclair.

h. Clonidine -- An Alpha-Adrenergic Agonist. Clonidine is administered intravenously to anxious and affectively ill patients and normal volunteers to assess clinical, physiological, and neuroendocrine responses to this noradrenergic drug. These studies are conducted in collaboration with Drs. G. Gurguis, W. Kaye, R. Post, L. Siever, and B. Vittone.

i. Yohimbine -- An Alpha-Adrenergic Antagonist. Yohimbine is administered in an oral challenge to panic anxious and affectively ill patients and normal controls to assess the clinical and biochemical effects of this noradrenergic antagonist which is known to potently increase noradrenergic function in the animal. These studies are conducted in collaboration with Drs. M. Albus, G. Gurguis, B. Vittone, T. Zahn, and with M. Geraci.

j. Nifedipine -- A Calcium Channel Blocker. Nifedipine is administered orally to agoraphobic patients exposed to nonphobic and phobic situations to determine the antianxiety effects, if any, of calcium channel blockers. This study is conducted in collaboration with Dr. E. Klein and M. Geraci.

k. Plasma MHPG, HVA, and Urinary Free Cortisol. Amine metabolites and urinary free cortisol are systematically evaluated using daily 24-hour urine collections across clinical state changes on and off medication. These studies are conducted in collaboration with Drs. G. Gurguis, D. Jimerson, M. Linnoila, and W. Potter.

l. Dexamethasone Suppression Test. Dexamethasone is administered to patients to evaluate the hypothalamic-pituitary-adrenal axis. Basal values are performed at baseline and at 4:00 pm, following dexamethasone administration.

m. Urine and Plasma Studies. Amine metabolites, electrolytes, and peptides are also measured in the urine and blood.

n. Alpha-Adrenergic Receptors. In collaboration with Dr. M. Kafka, platelet alpha receptor function as well as prostaglandin-stimulated increases in cyclic-AMP are assessed in patients and age-matched normal volunteers.

o. Platelet Imipramine Binding. In collaboration with Dr. W. Berrettini, [³H]imipramine binding to platelets is measured in patients and normal controls.

3. Treatment

a. Psychotherapeutic. Treatment and evaluation are conducted in individual and/or group supportive sessions. In addition, ongoing clinical case conferences are utilized. Collaborators in these studies include Drs. E. Klein, T. Mellman, B. Vittone, G. Gurguis, M. Stein, and M. Geraci and B. Scupi.

b. Routine Somatic Treatment. Both routine and experimental compounds are evaluated during double-blind clinical trials. Ongoing clinical trials include calcium channel blockers, tricyclic antidepressants, monoamine oxidase inhibitors, and minor tranquilizers. These studies are performed in collaboration with Drs. E. Klein, T. Mellman, B. Vittone, M. Stein, G. Gurguis, and M. Geraci and B. Scupi.

C. Major Findings (Studies in Humans):

1. Medical Illnesses and Anxiety

Detailed physical, neuropsychiatric, and laboratory evaluations continue to be performed in patients admitted to our program. As reported in previous years (Z01 MH 00071-04/05/06 BP), 60% of our panic patients had previously undiagnosed medical illnesses. Although these illnesses appeared to be unrelated to the direct pathogenesis of the panic attacks themselves, these data extend previous research suggesting that psychological (major life events) and physiological (medical illnesses) stressors may trigger panic attacks in biologically vulnerable individuals.

In an attempt to further define the characteristics of brain structure in panic disorder, we continue to investigate the cerebral ventricular size (VBR) in agoraphobic patients with panic attacks. The mean VBR in our patients was $3.4 \pm$

2.4 SD (range 1.0-9.0). Male (M) and female (F) patients had similar VBR (M: 4.0 ± 2.8 versus F: 2.9 ± 1.8 ; $t = 1.21$, $df = 23$, $p = NS$) and were of similar age at the time of the scan (M: 34.1 ± 6.5 versus F: 36.2 ± 7.0 ; $t = 0.77$, $df = 23$, $p = NS$). Of the 25 scans, one was read as clinically abnormal in a patient with abnormally small ventricles for age. VBR for patients who had a history of major depression ($n = 7$, 28%) (2.9 ± 1.5) or severe agoraphobia ($n = 7$, 28%) (3.2 ± 1.5) did not differ from the patients without a history of depression (3.6 ± 2.6 , $t = 0.65$, $df = 23$, $p = NS$) or severe agoraphobia (2.6 ± 3.5 , $t = 0.28$, $df = 23$, $p = NS$). There was a significant inverse relationship between VBR and duration of illness ($r = -0.55$, $p < 0.01$). There was no significant association between VBR and age ($r = -0.06$, $p = NS$), panic attacks within the past month ($r = 0.04$, $p = NS$), or state anxiety ($r = 0.19$, $p = NS$).

There was a significant association between VBR and duration of benzodiazepine use ($r = 0.51$, $p < 0.02$) and percent of time ill treated with benzodiazepines ($r = 0.67$, $p < 0.001$), although the mean VBR (3.8 ± 2.5) of the panic disorder patients who had received benzodiazepine treatment was similar to the patients without previous benzodiazepine exposure (2.5 ± 1.6 ; $t = 1.26$, $df = 23$, $p = NS$).

The findings, published in the J. Affective Disord., suggest that the ventricular size of panic disorder patients falls well within the normal range compared with reported values of mean VBR in normal control groups in the literature.

The nature of the relationship between VBR and duration of benzodiazepine exposure ($r = 0.51$, $p < 0.02$) in our study remains unclear but might be related either to a direct or indirect drug effect or merely be an artifact reflecting a tendency towards greater drug use in a subpopulation of patients with more severe illness.

2. Psychosensory Symptoms

The Unit continues to investigate the role of psychosensory symptoms in the phenomenology and longitudinal course of panic disorder. As noted in Z01 MH 00071-05/06 BP, panic disorder patients experience increased psychosensory symptoms during episodes of illness. During well intervals, the number of psychosensory symptoms in panic patients is similar to normal controls, although both nondepressed panic disorder patients and affectively ill patients report comparable increases in total number of psychosensory symptoms during clinical relapses. Since psychosensory phenomena occur spontaneously and after stimulation of the amygdala and hippocampus in patients with psychomotor epilepsy, these findings support an involvement of temporo-limbic structures in these psychiatric conditions. Of interest, preliminary findings suggest that the number and type of psychosensory symptoms does not predict clinical response to a wide variety of psychotropic medications.

3. Life Events and Onset of Panic Disorder

The life course of panic disorder continues to be assessed retrospectively in all patients with panic attacks. As reviewed in greater detail in

previous reports (Z01 MH 00071-04/05/06), several conclusions can be made regarding the phenomenology and longitudinal course of panic disorder. First, the onset of panic attacks generally begins in adolescence or early adulthood and, if untreated, frequently leads to an impaired life style characterized by pathological degrees of anticipatory or free-floating anxiety and agoraphobia. Second, lifetime symptoms of major depression occur in approximately 50% of the patients, although only 25% of all panic disorder patients develop longstanding endogenous symptoms of depression. Third, tricyclic antidepressants appear to have antipanic effects independent of the presence of concomitant depressive symptomatology.

During the past year, the Unit has focused on the role of life stressors and life events on the onset and course of illness of panic disorder. In order to evaluate the role of life events in the onset of panic disorder, we explored the number, type, and effect of life events occurring prior to the first panic attack in patients with panic disorder compared with similar data obtained in normal control subjects. Results of this study, reported in the American Journal of Psychiatry, indicate that panic disorder patients experienced more life events directly involving them. Comparisons by category, however, yielded differences only for events related to work and health. In particular, patients did not have more "exit" events than controls. Although there were no differences in objective degree to life change or stress, the patients did report greater subjective "distress".

These results suggest that patients with panic disorder, prior to the onset of their illness, personally experience more life events "happening to them" than controls. More importantly, life events seem to have a more adverse subjective effect on patients.

The major difficulty with this type of retrospective analysis involves the possible distorting effect of time on recall. Our analysis failed, however, to find an interaction between recall time and subject group by ANOVA. Using linear statistical analyses, the effects of time on recall was assessed in panic disorder patients and normal controls.

Not unexpectedly, time affected the number of events reported. However, there was no difference, in terms of the influence of time on the recall of each type of event, between the groups. The theoretical rationale for this statistical analysis and methodological implications of these findings were published in the Journal of Affective Disorders.

4. Life events and Course of Panic Disorder

The previous study indicated that an increased frequency of life events precedes the onset of panic disorder. Although there was no difference between panic disorder patients and normal controls in the total number of exit events, it remained unclear whether those patients who did experience a major separation or loss (e.g., death of spouse) prior to the onset of their illness would have a higher prevalence of subsequent major depressive episodes, panic attack frequency, or onset of agoraphobia.

Thirty-three patients with panic disorder participated in the study. They ranged in age from 21 to 45 years (mean \pm SD; age = 31.4 \pm 6.5 years), and their duration of illness ranged from 1 to 16 years (mean \pm SD = 6.1 \pm 4.8 years). In all cases, panic attacks were the first psychiatric symptom these patients had experienced and constituted the complaint that brought them to psychiatric attention.

Life events were determined using the Peri-M Events Scale. In this study we were only interested in the presence or absence of a major loss or separation, a type of event unlikely to be forgotten. Before we examined our data, we decided to define this type of event as including the death of a parent, sibling, or spouse, or the permanent dissolution of the patient's major attachment bond through divorce or another action (e.g., the breakup of a longstanding romantic relationship). There were 11 patients in the "major loss" subgroup, five having experienced the death of their father, two having been divorced, and four having faced the dissolution of a longstanding (longer than a year) romantic relationship. The remaining 22 patients had experienced other types of life events. Four patients who had undergone similar but less severe separation events (the death of an aunt, a transient separation from a mate, etc.) were excluded from the analysis.

Life course of illness was characterized with our life charting method. Variables extracted for this analysis included number of panic attacks in the year after onset, longest time free of panic attacks, rapidity of onset of agoraphobia, and presence or absence of a subsequent major depressive episode. In patients with a major depressive episode, we also recorded the time elapsed from the first panic attack to the episode, the severity of the episode, and the total number of episodes.

Parametric data were analyzed using a two-tailed Student's t test, and dichotomous data were analyzed using chi-square analysis. Patients with a severe loss preceding the onset of their illness had a significantly greater prevalence of subsequent major depression than did the no-loss group (85% versus 36%; $\chi^2 = 6.08$, $df = 1$, $p < .02$). There were no significant differences between groups in number of panic attacks, longest time free of panic attacks, or rapidity of onset of agoraphobia (45% within 3 months in the severe-loss group versus 36% in the no-loss group). For the majority of patients suffering a subsequent depression, these depressions were severe (78% in the severe-loss group and 78% in the no-loss group) and produced functional impairments regardless of whether or not there had been a severe loss preceding the onset of the illness. The time from first panic attack to the depression and the total number of lifetime depressions were similar whether or not there had been a prior loss.

These data, published in the American Journal of Psychiatry, suggest that the occurrence of major loss in patients with panic disorder confers an increased risk for a "secondary" depression without influencing the course of the primary anxiety symptomatology.

5. Panic Disorder: Relation with Obsessive Compulsive Symptoms

There has been much recent interest in the clinical and biological overlap of both panic and obsessive-compulsive disorders with major affective disorders. Although there is less known about the relationship between panic

disorder and obsessive-compulsive disorder, some evidence suggests a partial overlap in clinical phenomenology.

We determined the prevalence of obsessive-compulsive symptoms in our panic disorder population. We also compared clinical variables and treatment outcome in panic disorder patients with obsessive-compulsive symptoms to panic disorder patients without obsessive-compulsive features. Nineteen of 70 (27%) patients who met Research Diagnostic Criteria (RDC) for panic disorder reported obsessive-compulsive symptoms during a diagnostic interview utilizing the Schedule for Affective Disorders and Schizophrenia (SADS), anxiety disorders section. Of this group, 10 (53%) reported obsessional symptoms only, two (10%) reported compulsive symptoms only, and the remaining seven (37%) reported obsessional plus compulsive symptoms. These 19 patients with panic disorder (PD) plus obsessive-compulsive symptoms (OCS) comprised the study group (PD + OCS).

A comparison group without OCS was selected to form a homogeneous population with certain classic features of panic disorder. Followup data were obtained by a structured telephone interview of patients who had completed the NIMH treatment program a mean of 16.3 ± 13.4 months (range 3-46) for the PD + OCS group, and 13.4 ± 10.0 months (range 7-32) for the PD - OCS group prior to phone contact. Sixteen of 19 of the PD + OCS group (84%) and 14 of 25 of the PD - OCS group (56%) were available for the phone interview ($p = NS$). The interview included information on the three-month prevalence and self-rated change in the following items: panic, generalized anxiety, avoidance, obsessions, and compulsions. Patients made a global judgment as to whether each of these five symptoms had improved, were unchanged, or had worsened since their discharge from the program. The interview also included social disability scale.

Compared to the PD - OCS group, a lifetime history of major depression by DSM-III criteria was more frequent in the PD + OCS group ($p < .001$) as was a past history of alcohol or drug abuse ($p < .05$). The PD + OCS group patients had a significantly earlier onset of illness (20.3 ± 4.7 years) compared to patients without obsessive-compulsive symptoms (27.1 ± 7.7 ; $X^2 = 3.43$, $p < .01$). The two groups did not differ significantly with regard to the presence or severity of agoraphobia. There was no difference noted in the two groups for frequency of panic attacks, total years ill, or percentage of time in remission prior to the NIMH evaluation.

There was a significantly greater incidence of primary affective disorders (63% versus 20%) and alcoholism or substance abuse (47% versus 8%) in the first degree relatives of panic patients with obsessive-compulsive symptoms compared to the first degree relatives of patients without obsessive-compulsive symptoms. There was no difference in the incidence of panic or phobic disorders.

While both groups reported improvement in panic attacks, persistent attacks were more common at follow-up in the PD + OCS patients, even though 89% and 77% of the PD + OCS and PD - OCS groups, respectively, had greater than one panic attack per month at the time of initial evaluation. Fewer PD + OCS patients compared to PD - OCS patients reported "improvement" in generalized anxiety (6/16 versus 12/14, $X^2 = 5.36$, $p < .02$) and a greater number reported the persistence of moderate to severe generalized anxiety (13/16 versus 4/14, $X^2 = 6.3$, $p < .01$).

These data suggest that the presence of obsessive-compulsive symptoms in panic disorder may be a clinical predictor of a subgroup of panic disorder patients with distinct features and substantially less optimal treatment outcome, particularly in relation to symptom interference with functioning. The greater impairment in this subgroup cannot be attributed solely to the additional problems associated with a second separate neuropsychiatric disorder (i.e., obsessive-compulsive disorder), since these patients also reported a greater rate of panic attacks and more severe generalized anxiety at follow up.

6. Sleep, Sleep-related Panic, and Sleep Deprivation

In reports Z01 MH 00071-04/05/06 BP we reported in detail the findings on the sleep EEG of panic disorder patients. Since a major focus of the Unit's ongoing research is the investigation of the relation between panic and major depressive disorders, we were particularly interested in the nature of rapid-eye-movement (REM) parameters in patients with panic disorder compared to normal controls. Preliminary data from our laboratory, published in Psychiatry Research, suggested that panic disorder patients did not have marked reductions in REM latency typical of patients with melancholic depression. In fact, in this initial study, our panic disorder patients had a significantly lower REM density and a normal progression in the length of each successive REM period. These findings have been both confirmed and extended by our laboratory in a second separate study.

In our second study, we investigated the sleep EEG of patients with panic and major depressive disorders and normal controls. The sleep of the panic disorder patients was generally disturbed, as manifested by significant decreases in sleep time and sleep efficiency and increased sleep latency. These disturbances were more prominent in the panic disorder patients compared with both the depressed patients and normal controls. Preliminary findings also suggest that REM latencies are reduced in depressed patients compared to the panic disorder patients and normal controls.

Six of 13 patients experienced sleep panic attacks. The sleep panics were all characterized as sudden awakening with fear or apprehension, without recall of any specific dream content. The symptoms most commonly reported for these episodes included palpitations occurring during 100% of the episodes, sweating (67%), hot or cold flushes (50%), choking or smothering sensation (50%), feelings of unreality (50%), and chest pain or discomfort (50%). The attacks occurred between 24 and 225 minutes from sleep onset and between 65 minutes before and 48 minutes after the first REM period. The epoch preceding the awakenings with panic were scored as stage 2 for two of the six sleep panics and stage 3 for the remaining four attacks. Some EEG slowing preceded the awakenings from stage 2, and the awakenings from stage 3 were preceded by a maximum of only two minutes of stage 3 sleep. The amount of time from the awakening to resuming stage 1 or stage 2 sleep ranged from two to seven minutes.

Nights of sleep panic featured increased REM latencies (101.2 minutes \pm 40.4 versus 69.9 \pm 18.9, $p < .05$) and increased minutes of stage 3 sleep (26.2 \pm 26.5 versus 22.5 \pm 25.0, $t = 2.98$, $p < .05$) in comparison to non-panic nights. There was a trend for less movement time to occur on panic nights. These data suggest that panic attacks occurring from sleep are not an infrequent feature of panic

disorder and provide a potentially useful model for elucidating mechanisms of panic.

All six of the attacks recorded in this study occurred from non-REM sleep and, in comparison with other spontaneous awakenings, there appears to be some specificity for stage 3 sleep. In fact, all of the panic awakenings could be characterized as being preceded by a transition from a lighter to deeper stage of non-REM sleep; i.e., proceeding from stage 2 toward delta sleep.

That panic can occur in association with the progression toward a "deeper" stage of sleep is of interest with regard to the observation that increased basal arousal is often predictive of subsequent panic in many panic induction studies, such as those utilizing sodium lactate infusions. Our findings suggest that increased basal arousal is not a requirement for panic and that panic may actually occur in the context of diminishing arousal.

Several studies have documented that one night's total sleep deprivation is associated with a clinically robust but transient improvement in mood in depressed patients. In fact, patients with the more classic symptoms of melancholia appear to be the best responders to sleep deprivation, while more atypical depressives tend to be poor responders to sleep deprivation. On the basis of these observations, we hypothesized that nondepressed panic disorder patients would fail to respond positively to one night's total sleep deprivation.

The effects of one night's total sleep deprivation was studied, therefore, in panic disorder patients and compared with results in depressed patients and normal controls previously studied in our laboratory. As a group, the depressed patients demonstrated significantly more improvement in nurse-rated measures of anxiety than both the panic disorder patients and controls. Depressed patients showed a decrease, while panic patients had an increase in measures of anxiety, including some patients who experienced panic attacks. These findings, published in the Archives of General Psychiatry, suggest that both the sleep EEG and behavioral response to sleep deprivation are reliable markers in distinguishing between nondepressed panic disorder patients and patients with major depressive disorder, melancholic subtype.

7. Urinary Free Cortisol and Plasma MHPG in Panic Disorder

Alterations in noradrenergic function have been postulated to play an important role in the modulation of fear and anxiety. Moreover, the noradrenergic system appears to be functionally related to hypothalamic-pituitary-adrenal (HPA) axis function. The Unit on Anxiety and Affective Disorders, therefore, studied urinary free cortisol and plasma MHPG in 12 panic disorder patients and 12 normal controls.

There was no significant difference in either MUFC or plasma MHPG levels between panic patients and normal controls. Two of 12 patients versus none of 12 controls had a MUFC greater than the normal range of 9 to 95 $\mu\text{g}/24$ hrs. There was no significant correlation between plasma MHPG and MUFC in the total sample ($r = 0.27$, $df = 22$, $p = \text{NS}$) or in the panic patients ($r = 0.17$, $df = 10$, $p = \text{NS}$) or controls ($r = 0.52$, $df = 10$, $p = \text{NS}$) as separate groups.

Six patients reported a mean of 2.3 ± 1.6 (range 1-5) panic attacks during the three day period of the study (panic attack-positive patients [PA+]). The mean Spielberger anxiety ratings for the total patients group were 46.3 ± 6.3 . There was no significant difference in mean Spielberger anxiety ratings in PA-positive (49.2 ± 1.7) versus PA-negative (43.4 ± 8.1) patients. The PA-positive patients did not have significantly different plasma MHPG (3.2 ± 0.8 SD) or MUFC ($62.4 \mu\text{g}/24 \text{ hours} \pm 31.4$ SD) values compared with the PA-negative patients (MHPG: 4.0 ± 0.8 SD, $t = 1.66$, $df = 10$, $p = \text{NS}$; MUFC: $51.6 \mu\text{g}/24 \text{ hours} \pm 26.2$ SD, $t = 0.64$, $df = 10$, $p = \text{NS}$), normal controls (MHPG: 3.7 ± 0.9 , $t = 1.0$, $df = 16$, $p = \text{NS}$; MUFC: 52.7 ± 18.8 , $t = 0.82$, $df = 16$, $p = \text{NS}$), or the combined group of PA-negative plus normal controls (MHPG: 3.8 ± 0.9 SD, $t = 1.39$, $df = 22$, $p = \text{NS}$; MUFC: $52.4 \mu\text{g}/24 \text{ hours} \pm 20.8$ SD, $t = 0.90$, $df = 22$, $p = \text{NS}$).

There were no significant correlations between MHPG and frequency of panic attacks ($r = -0.46$, $df = 10$, $p = \text{NS}$) or measures of state anxiety ($r = -0.40$, $df = 10$, $p = \text{NS}$), global anxiety ($r = -0.32$, $df = 10$, $p = \text{NS}$), agoraphobia ($r = -0.32$, $df = 10$, $p = \text{NS}$) or depression ($r = 0.11$, $df = 10$, $p = \text{NS}$) in the patients. There was also no significant correlation between MUFC and frequency of panic attacks ($r = 0.35$, $df = 10$, $p = \text{NS}$) or measures of state anxiety ($r = -0.31$, $df = 10$, $p = \text{NS}$), global anxiety ($r = -0.06$, $df = 10$, $p = \text{NS}$), agoraphobia ($r = 0.49$, $df = 10$, $p = \text{NS}$) or depression ($r = 0.27$, $df = 10$, $p = \text{NS}$) in the panic disorder patients.

Our MUFC data suggest that increased HPA axis function is not a prominent feature of panic disorder. Our Unit has reported, however, that patients with panic disorder have elevated evening plasma cortisol levels and reduced ACTH and cortisol responses to corticotropin releasing hormone (CRH) (see 15C). Similar findings with the CRH test have been reported in depressed patients. These observations suggest that while panic patients may not have persistently elevated indices of increased HPA function, there may be discrete periods of hypercortisolemia associated with the illness. Mean plasma MHPG values did not differ between panic patients and controls.

We also failed to find a relationship between MHPG and frequency of panic attacks or ratings of global anxiety or agoraphobia. These observations and our finding that plasma MHPG did not distinguish PA-positive from PA-negative patients are consistent with the suggestion that noradrenergic overactivity is not a biological pre-requisite for all panic attacks or other types of pathological anxiety. Moreover, although an association between adrenergic activation and plasma cortisol have been reported in depressed patients, we found no correlation between plasma MHPG and urinary free cortisol in either panic disorder patients or normal controls. These observations, to be published in *Biological Psychiatry*, further suggest that the mechanisms underlying central adrenergic and peripheral HPA activation may have different dimensions of functional relatedness, depending on the nature and state of the psychiatric syndrome.

8. Plasma HVA in Panic Disorder

To assess dopamine function in panic disorder, plasma HVA and MHPG was investigated in 15 panic disorder patients and nine normal controls. As reported in Z01 MH 00071-06 BP, we found no difference in plasma HVA between panic disorder patients and normal controls. However, plasma HVA values showed a bimodal distribution. Comparisons of high and low HVA subgroups were tabulated. Patients in the "high" HVA subgroup, compared with those in the "low" subgroup, had significantly higher Spielberger State Anxiety scores, more panic attacks in the previous year, and shorter maximal time free of panic attacks.

These findings, published in Biological Psychiatry, suggest that panic disorder patients with higher concentrations of plasma HVA are more anxious, have increased panic attacks, and have shorter symptom-free remissions. Although the panic disorder patients did not as a group have higher HVA compared to normal controls, these preliminary findings suggest a possible role for dopamine systems in the neurobiology of panic disorder.

9. Effects of Diazepam on Mood, Memory, and Pain

The effects of clonidine on the somatosensory pain threshold have been discussed in previous annual reports (Z01 MH 00071-04 and Z01 MH 00071-05). The Unit has expanded these studies to include the assessment of the effects of diazepam on mood, memory, and pain.

Using previously described methods (Z01 MH 00071-04/05), the Unit recently found that 10 mg of diazepam produces a significant impairment in effortful memory and attention, but had no effect on automatic memory, semantic memory, or judgment memory. These findings were published in Psychopharmacology. Data also suggest that diazepam has a subtle analgesic effect due to its ability to prevent the normal improvement in discriminability associated with somatosensory retesting. Although diazepam produced mild-moderate subjective effects such as the induction of "lethargic", "dreamy", "drowsy", and "muggy-headed" states, none of these subjective feelings was related to diazepam's effects on free-recall and attention. Overall, the effects of diazepam on pain and memory appear to be a separate phenomenon. These findings, together with previous data (Z01 MH 00071-04/05) showing that pain and anxiety may have opposite relationships in panic disorder patients compared to normal controls, suggest that diazepam may have differential effects on pain sensitivity in patients with anxiety disorders.

10. Effects of Diazepam on Cortisol and Beta-endorphin

Benzodiazepines have been shown to have neuroendocrine effects in both animals and humans. The most consistently observed endocrine changes in response to benzodiazepines have been decreases in ACTH and cortisol. Increases in growth hormone (GH) have been observed by some but not all investigators. Numerous studies have demonstrated that various kinds of stress can activate the HPA axis, causing increases in both cortisol and ACTH. Benzodiazepines have also been shown to antagonize stress-induced increases in both ACTH and cortisol in animals and humans.

The neuroendocrine effects of 5 and 10 mg of orally administered diazepam were, therefore, assessed in ten normal subjects under baseline (pre-stress) and laboratory-controlled stressful conditions. Although the 10 mg diazepam dose had no effect on cortisol at baseline, it significantly reduced the increase in cortisol produced by 15 minutes of exposure to a painful electrical stimulus. There were no significant effects on growth hormone, beta-endorphin, or ACTH either at baseline or following painful stimulation.

Our results, to be published in the Journal of Clinical Psychopharmacology, document the ability of diazepam to blunt stress-induced increases in plasma cortisol in normal subjects. These results suggest the possibility of employing the cortisol-response to diazepam in assessing the function of the benzodiazepine system in different psychiatric conditions.

11. Caffeine: Behavioral and Biochemical Effects

Several studies have been conducted by the Unit on Anxiety and Affective Disorders to investigate the behavioral and biochemical effects of caffeine in panic disorder patients and normal controls. The following section reflects the scientific rationale and chronological sequence of our research with caffeine.

a. Caffeine: Retrospective Survey. As previously reported (Z01 MH 00071-04/05/06), a caffeine-consumption survey was designed and administered to patients with panic and major depressive disorders and compared to normal controls matched for age, sex, and socioeconomic status. Data from this survey, published in Psychopharmacology Bulletin and the Archives of General Psychiatry, indicated an increased sensitivity to the psychostimulant and anxiogenic effects of caffeine in panic disorder patients compared to their normal controls. This relationship was not found in patients with major affective disorders. The findings of this survey, suggesting an increased vulnerability to the anxiogenic effects of caffeine in patients with panic disorder, led us to directly test the single-dose behavioral and biochemical effects of caffeine in panic disorder patients and normal controls. To pursue this goal, our Unit first investigated the effects of three separate doses of caffeine in normal controls.

b. Caffeine: Effects on Anxiety, Blood Pressure, Lactate, and Cortisol in Normal Controls. Using double-blind, placebo-controlled conditions, three doses of oral caffeine (240, 480, and 720 mg) were administered to 14 normal controls. Caffeine produced dose-related increases in state anxiety, mean arterial pressure, plasma lactate, and plasma cortisol. Plasma NE and its principal metabolite, MHPG, failed to increase. Two of 14 normal controls developed unequivocal panic attacks following the 720 mg dose of caffeine. This research demonstrated that caffeine in sufficient doses may induce anxiety, including panic attacks, in normal subjects. The lack of caffeine's effects on MHPG further suggested that noradrenergic systems might not be responsible for the major psychostimulant effects of caffeine in euthymic humans.

c. Caffeine: Effects on Plasma Adenosine Levels. Using the beforementioned design (Section C, 10b), plasma adenosine was measured in normal volunteers. The measurement of plasma adenosine after oral caffeine in humans was

investigated since caffeine-induced behavioral changes in animals are thought to be mediated by blockade of adenosine receptors. In a subgroup of eight normal volunteers presented in Section C, 10b of this report, three oral doses of caffeine (240, 480, and 720 mg) and placebo were administered on four separate occasions. Adenosine levels were determined as described in Section B, 2g. Despite dose-related increases in anxiety and plasma caffeine levels (up to 73.3 μM), no significant change in plasma adenosine concentrations was observed after caffeine administration. Although plasma adenosine levels did not change, these data support a role for adenosine receptor systems in caffeine-induced anxiety states since the caffeine levels reached after administration of 720 mg, the only dose which in this small sample produced significant anxiogenesis, are in a range (44-73 μM) known to compete with the binding of various ligands to the adenosine receptors in human brain (Ki-35-115 μM).

d. Increased Sensitivity to Caffeine in Panic Disorder Patients.

To directly test our hypothesis that panic patients have an increased vulnerability to the anxiogenic effects of caffeine, a caffeine dose (480 mg) which failed to elicit panic attacks or severe degrees of generalized anxiety in the normal controls, was administered under double-blind, placebo-controlled conditions, to 24 panic disorder patients and compared to the 14 normal controls reported in 11b. The results of this study support our hypothesis of increased sensitivity to caffeine in panic patients, as indicated by a significantly greater increase in measures of anxiety on the Zung Anxiety Scale in the patients compared to normal controls. Moreover, nine of 24 panic patients, but none of the 14 normal controls experienced panic attacks by DSM III criteria. Compared to normal controls, the panic patients also had significantly higher levels of cortisol, lactate, and glucose following caffeine, although only increased levels of lactate distinguished between panicking and nonpanicking patients. It should be underscored that the normal controls did have significant increases in both measures of anxiety and plasma cortisol, compared to their placebo control condition. Thus, while panic patients appear more sensitive to the anxiogenic effects of caffeine, normal subjects are not insentient to the psychostimulant properties of caffeine.

e. Alprazolam Blocks Anxiogenic Effects of Caffeine. We have conducted preliminary studies investigating the effects of alprazolam, a triazolabenzodiazepine with antipanic properties in humans, on caffeine-induced anxiety. Blinded caffeine 480 mg was administered to patients participating in a double-blind, alprazolam-placebo crossover study. While six of 16 (37.5%) on the placebo phase of the study had panic attacks following single dose caffeine (480 mg), none of 11 (0%) of the alprazolam-treated patients had panic attacks following this same acute oral dose of caffeine ($p = .027$, Fisher's exact test). Of interest, alprazolam blocked the usual caffeine-induced increment in lactate but had no effect on plasma cortisol levels. These behavioral and biochemical effects suggest that the benzodiazepine receptor system may play an important role in blocking some of caffeine's psychostimulant and biochemical effects. The role of the GABA-benzodiazepine receptor system in mediating caffeine's principal panicogenic effects remains to be elucidated.

f. Caffeine-induced Escape from Dexamethasone Suppression. The dexamethasone suppression test (DST) has been suggested as a sensitive and specific tool for the diagnosis of major depressive disorder, melancholic subtype.

Because psychiatric patients have been reported to consume excessive amounts of caffeine, because caffeine produces dose-related increases in plasma cortisol (refer to Sections C, 10b and 10c of this report), and because the effects of caffeine (probably the most widely consumed psychotropic agent the world) on the DST had not been previously reported in the literature, the single-dose effects of caffeine 480 mg on the standard dexamethasone suppression test was investigated in 23 normal volunteers, 13 depressed and two panic disorder patients. Using a single-blind design, an oral dose of caffeine 480 mg or placebo was administered randomly on two separate days at 2:00-2:30 pm the day following the 11:00 pm administration of dexamethasone 1 mg. Test days were separated by at least 48 hours. Blood samples were obtained at 4:00 pm.

Caffeine significantly increased the post-dexamethasone cortisol values. Whereas the 4:00 pm cortisol values after placebo averaged 2.3 ± 2.3 (mean \pm SD), the comparable mean value after caffeine was 5.3 ± 5.8 (paired $t = 3.7$, $p < .001$). A plasma cortisol level of $> 5 \mu\text{g/dl}$ has been used most commonly to signify non-suppression. Of the 38 subjects, five (13%) were found to be nonsuppressors on placebo and 12 (31%) were nonsuppressors on caffeine. Caffeine-induced nonsuppression was observed in both depressed patients and normal volunteers. This study is the first investigation to our knowledge demonstrating that escape from dexamethasone suppression can be induced by caffeine. Of interest, the 480 mg single dose of caffeine given to subjects in this study is roughly comparable to four to five cups of coffee and within the range typically consumed on a daily basis by 20%-40% of the population. Since several lines of evidence suggest that psychiatric patients, particularly depressed and schizophrenic patients, may consume excessive amounts of caffeine, our findings may explain in part the wide variability and discrepant findings in the literature on the DST in psychiatric patients.

12. GH-response to Clonidine

Studies using clonidine to assess noradrenergic function continue to be investigated by the Unit on Anxiety and Affective Disorders. Several lines of evidence, reviewed in previous reports (Z01 MH 00071-04 BP and Z01 MH 00071-05 BP), suggest that the GH-response to clonidine may provide an index of postsynaptic α_2 -adrenergic function. Since noradrenergic dysfunction, particularly noradrenergic overactivity, represents one of the major current theories of anxiety, the GH-response to clonidine was studied in nondepressed panic disorder patients compared to depressed patients and normal controls triple-matched for age, sex, and menstrual cycle status.

The mean peak growth hormone response to clonidine was significantly decreased in the panic disorder patients ($n = 11$, $x = 1.8 \pm 1.2$ SD) and depressed patients ($n = 11$, 3.8 ± 4.3) compared to normal controls ($n = 11$, 12.7 ± 11.7) ($F = 7.91$, $p < 0.002$). This finding remained significant when men ($n = 4$, $F = 20.48$, $p < 0.0004$), women ($n = 7$, $F = 4.51$, $p < 0.03$), and the subgroup of patients (four men and three women) without elevated baselines ($F = 6.31$, $p < 0.008$) were analyzed as separate groups. There was no significant difference in baseline GH levels among the three groups.

Our findings, published in *Biol. Psychiatry*, suggest that panic disorder and depressed patients demonstrated a similar blunted growth hormone response to

clonidine compared to age- and sex-matched normal controls. These findings are consistent with an emerging body of data suggesting a partial, but incomplete, overlap in the phenomenology, epidemiology, and neurobiology of panic and major depressive disorders.

13. Cortisol-response to Clonidine

Abnormalities in regulation of noradrenergic function have been proposed as part of the pathology of depressive and panic anxiety disorders. However, abnormalities in HPA axis function have largely been limited to patients with depressive disorders. Using the cortisol response to clonidine, an alpha₂-adrenergic receptor agonist, this study examined the relationship between the noradrenergic system and the HPA axis in ten patients with major depression (4 unipolar, 6 bipolar), ten patients with panic disorder, and ten normal controls.

There was a trend for the three diagnostic groups to differ in baseline cortisol values prior to infusion (panic: 6.1 ± 4.7 $\mu\text{g/dl}$; depressed: 12.6 ± 6.2 $\mu\text{g/dl}$; controls: 9.6 ± 6.9 $\mu\text{g/dl}$; $F = 2.98$, $p < .07$). The significant difference was between the depressed and panic groups ($p < .02$).

The mean fall in plasma cortisol after clonidine was 1.7 ± 2.4 $\mu\text{g/dl}$, 5.2 ± 4.9 $\mu\text{g/dl}$, and 2.8 ± 2.8 $\mu\text{g/dl}$ in the panic disorder ($p < .06$), depressed ($p < .01$), and normal controls ($p < .02$), respectively. There was a trend for the three groups to differ in the fall in plasma cortisol in response to clonidine ($F = 2.56$, $p < .10$), with the trend seen between the depressed and panic groups ($p < .06$). However, when the fall in plasma cortisol was expressed as a percentage change from baseline, there was no significant difference among the three groups (panic: $26.2 \pm 38.6\%$; depressed: $38.8 \pm 17.7\%$; controls: $31.3 \pm 18.3\%$; $F = .563$, $p > .10$).

The pre-clonidine plasma cortisol level was significantly negatively correlated with the absolute clonidine-induced change in cortisol level in the depressed patients ($r = -0.87$, $df = 8$, $p = < .005$), in the controls ($r = -.75$, $df = 8$, $p = < .02$), and in the panic patients ($r = -0.77$, $df = 8$, $p = < .001$). The composite correlation for the group of 30 patients was: $r = -.81$, $df = 28$, $p < .0001$. However, the pre-clonidine plasma cortisol level did not correlate significantly ($p > .10$) with the percentage of change in plasma cortisol level in any of the three groups.

This is the first study to compare the cortisol response to clonidine across subjects with panic disorder, major depression, and normal controls. While the enhanced cortisol drop following clonidine might suggest a difference in noradrenergic modulation of the HPA axis in depression compared to panic disorder, we believe that the inhibitory effects of clonidine on cortisol secretion may be a less than satisfactory probe of this relationship.

The apparent greater drop in cortisol may simply be a function of higher baseline cortisol levels in the depressed group. We found that the drop in cortisol was highly correlated with the baseline cortisol level; when a percentage, rather than absolute, drop was measured, this correlation was not seen. Accordingly, the percentage drop in cortisol, a measure independent of

baseline cortisol level, did not differ across diagnostic groups. Because of the dependence on baseline cortisol levels, the absolute drop in cortisol in response to clonidine may provide little information about α_2 -adrenoreceptor sensitivity. These findings, and other data to be published in Biological Psychiatry, have led us to conclude that "stimulatory" rather than "inhibitory" behavioral and neuroendocrine responses may be more reliable paradigms for the study of neurochemical-neurotransmitter function in panic disorder.

14. Panic Disorder: Platelet Imipramine Binding

The nature of the relationship between panic and major affective disorders is currently a subject of scientific controversy. The favorable response of patients with panic disorder to tricyclic and monoamine oxidase inhibitor antidepressants, the high prevalence of major depressive episodes in panic disorder patients, and the greater frequency of depression in relatives of depressed patients with concomitant panic disorder compared to relatives of depressed patients without panic disorder, suggest an overlap between panic disorder and major depression. Similar neuroendocrine responses to clonidine also suggest a neurobiological relationship between the two disorders. Several lines of evidence, however, suggest important differences between the two disorders. Since most studies have reported that the number of binding sites on blood platelets are reduced in depressed patients compared to normal controls, we investigated platelet imipramine binding in panic disorder patients.

[³H]Imipramine binding to platelets was measured in 17 drug-free panic disorder patients and 14 healthy controls. No difference in E_{max} or K_d values was found between the two groups. Patients with a past history of major melancholic depression or severe agoraphobia had binding parameters similar to those of panic disorder patients without a history of depression or severe agoraphobia. Thus, our findings, published in Biological Psychiatry, suggest that nondepressed panic disorder patients, with or without a past history of endogenous depression or agoraphobia, may not display a lower density of platelet [³H]imipramine binding sites compared to normal controls, as has been reported in many depressed patients.

15. Panic Disorder and Neuroendocrine Function

The Unit on Anxiety and Affective Disorders has investigated several neuroendocrine tests in panic disorder patients. With the exception of new data presented in 15a, the results of these ongoing investigations were presented in more detail in last year's report (Z01 MH 00071-06 BP) and are only briefly summarized here.

a. T₃, T₄, and TSH Levels. Anxiety is a common manifestation of hyperthyroidism and thyroid disorders may mimic the clinical presentation of panic disorder. What is less clear from the literature is whether or not panic disorder, in the absence of clinically overt thyroid disease, is routinely associated with physiologic disturbances in thyroid function. Using a closely age- and sex-matched normal control group, we were able to explore the hypothesis that patients with panic disorder might exhibit subtle abnormalities in routine indices of thyroid function. The samples studied consisted of 26 panic disorder patients, and 26 closely age- and sex-matched normal volunteers. There were no

significant differences in measurements of T_3 , T_4 , FT_4 , TSH, or TBG between patients with panic disorder and normal controls. When these values were compared separately by sex, no differences emerged. Furthermore, our findings do not rule out the possibility that hypothalamic-pituitary modulation of peripheral thyroid function may be altered in panic disorder.

This study, to be published in the *American Journal of Psychiatry*, suggests that abnormalities in baseline and peripheral indices of thyroid function are not a requisite biological correlate of panic disorder. That abnormal T_3 , T_4 , FT_4 , and TSH levels were not found in our panic disorder patients does not preclude the possibility that patients with primary thyroid dysfunction might be predisposed toward the secondary development of anxiety syndromes, even after correction of an underlying thyroid disorder.

Our Unit had previously speculated that the blunted TSH response to TRH might reflect a condition of subclinical hyperthyroidism in panic disorder. However, given our new findings presented here, it seems less likely that the blunted TSH response is a consequence of subclinical hyperthyroidism, since we found no tendency for panic disorder patients to exhibit evidence of excess peripheral thyroid hormone levels.

b. Thyroid Releasing Hormone (TSH) Test. A TRH test was administered to 12 patients with panic disorder and ten normal volunteers. A Bmax TSH of less than 7 U/ml was used as the criterion for a blunted TSH response. Four of 12 panic disorder patients and none of the ten controls demonstrated a reduced TSH response to TRH ($p = .06$). In addition, the panic patients had a significantly lower Bmax TSH value compared to the normal controls. These findings, published in the *American Journal of Psychiatry*, suggest that the blunted TSH response to TRH may be common to both panic disorder and major depressive disorders and represent inappropriate hyporesponsiveness of the thyrotrope.

c. Corticotropin Releasing (CRH) Test. A CRH test was performed on eight panic disorder patients and compared with 27 normal controls previously studied by the Biological Psychiatry Branch. Compared with normal controls, panic disorder patients had decreased ACTH responses ($p < .01$) and reduced cortisol responses ($p < .05$) to CRH. These findings, published in the *Am. J. Psychiatry*, suggest that panic disorder patients may have an element of chronic hypercortisolemia and an abnormality in CRH secretion similar to that proposed for depressed patients. In addition, of the two baseline ACTH values obtained prior to the CRH challenge, the mean initial ACTH value was significantly higher than the second value. In control subjects, the two basal ACTH values were not significantly different. This initial elevated basal ACTH level in the panic disorder patients suggests a more acute perturbation in CRH secretion, one not seen thus far in any other group of hypercortisolemic psychiatric patients tested. The possibility that patients with panic disorder might more readily release CRH in response to environmental perturbation is intriguing in light of the animal data documenting that intracerebroventricular administration of CRH produces a variety of behavioral and physiological changes classically associated with the stress response. Finally, the ability of CRH to increase both locus coeruleus activity and plasma norepinephrine levels is provocative in light of theories implicating increased central noradrenergic activity in the etiopathology of panic disorder.

d. Dexamethasone Suppression Test. A standard dexamethasone suppression test was administered to 16 panic patients and 22 normal controls. Using a standard cortisol value of greater than 5 µg/dl to indicate nonsuppression, there was no significant difference between the proportion of panic patients (25%) and normals (14%) with an abnormal test. Moreover, an internal standard for the dexamethasone suppression test was determined using our normal control data. Using a 95% confidence interval (mean + 2 SD) as the criterion for abnormal response (7.1 µg/dl), only 6% and 4% of the panic patients and normal controls, respectively, demonstrated cortisol escape from dexamethasone suppression. Our results, published in Biological Psychiatry, indicate that panic patients do not respond abnormally to dexamethasone testing when a control group is used to determine the range of normality for a given assay and testing condition.

16. Alprazolam Withdrawal

Alprazolam is an effective and widely-used benzodiazepine in the treatment of panic disorder. A potential disadvantage of alprazolam, however, is the development of withdrawal symptoms following the abrupt discontinuation of relatively high doses. Therefore, the Unit investigated both the clinical and biological correlates of gradual alprazolam withdrawal and the utility of carbamazepine in the treatment of alprazolam withdrawal.

a. Behavioral and Biological Correlates. In the first study, ten patients (five men and five women, mean age 31.0 ± 8.7) maintained on an average of $4.95 \text{ mg} \pm 3.22 \text{ S.D.}$ alprazolam (range 1.0 to 12.0 mg) for 4 to 22 months ($x = 11.7 \pm 5.8$) were studied during alprazolam withdrawal on the 3-West inpatient unit. For the purposes of this study, the behavioral and biological indices of the "withdrawal" period were compared to a stable "post-withdrawal" period.

Spielberger anxiety ratings were found to be significantly elevated during the "withdrawal" compared to the "post-withdrawal" period. The cortisol values were consistently more elevated during alprazolam withdrawal than when medication-free. During "withdrawal" as compared to "post-withdrawal", there was also a trend for increased pulse rates and systolic blood pressure. Significant differences in diastolic blood pressure, temperature, and hours of sleep were not demonstrated. There was a significant correlation between changes in (withdrawal minus post-withdrawal) cortisol and pulse values.

These findings, published in the American Journal of Psychiatry, suggest that increased measures of anxiety and plasma cortisol are commonly associated with gradual tapering of relatively low doses of alprazolam.

b. Treatment. In an attempt to explore withdrawal modifying strategies, a preliminary study investigating the utility of carbamazepine in the treatment of alprazolam withdrawal has been initiated by the Unit on Anxiety and Affective Disorders. Three patients who had extreme difficulties during blind withdrawal from alprazolam demonstrated during a second withdrawal phase that they were able to tolerate a comfortable and more rapid alprazolam withdrawal when treated with carbamazepine than without this agent. These preliminary findings, published in the American Journal of Psychiatry, suggest that carbamazepine might provide a potentially useful therapeutic tool in the treatment of benzodiazepine withdrawal.

17. Panic Disorder: Treatment

a. Verapamil. Calcium channel blocking agents are widely used in the treatment of cardiovascular disorders. Recent evidence also suggests that these drugs might have positive therapeutic effects in patients with major affective disorders. In a double-blind, placebo-controlled study, we investigated the effects of verapamil, a calcium channel blocker, in the treatment of panic disorder. The study was designed as a double-blind, crossover study and each patient received both verapamil and placebo.

Each patient first received four weeks of placebo treatment. After the first placebo period, verapamil was initiated at a dose of 160 mg/day and increased by 160 mg at weekly intervals to a maximum dose of 480 mg/day. This dose was maintained for five weeks. The dose was then decreased to 240 mg/day for one week prior to discontinuation and placebo substitution for an additional four weeks. Thus, the study was an off-on-off design lasting a total of 16 weeks. Analysis of variance with repeated measures was employed comparing the pretreatment placebo period versus treatment period versus post-treatment placebo period with the corrected Huynhfeldt probability; posthoc analyses were performed employing the Tukey test.

Eleven patients completed the study. A one-way repeated measure analysis of variance (ANOVA) (first placebo versus active drug versus second placebo periods) revealed a significant drug effect on anxiety as measured by the Zung anxiety scale (mean ratings: 58 ± 10.5 , 51.9 ± 11.1 , 51.5 ± 10.6 , respectively, $F = 4.19$, $df 1.6/16.1$, $p < 0.04$). Posthoc analysis revealed a significant difference between Zung ratings during the placebo pretreatment period and the ratings in the post-treatment period ($p < 0.05$) with a similar trend between the "pretreatment" and the "on-treatment" values ($p < 0.06$). However, there were no significant changes on the Spielberger state anxiety, the NIMH agoraphobia, and the Beck depression scales. Nine of 11 patients (82%) had a decrease in the number of panic attacks during the last four weeks of verapamil treatment compared to the four weeks on placebo preceding verapamil ($p < 0.02$, Signed Rank test).

These findings, to be published in the Am. J. Psychiatry, suggest that verapamil has modest anxiolytic and antipanic effects. Additional studies are required to substantiate this finding and compare the efficacy of verapamil to that of drugs such as imipramine, phenelzine, and alprazolam which have a well-established role in the treatment of panic disorder.

b. Carbamazepine. Our findings of a high frequency of psychosensory symptoms (see C2) and electroencephalographic abnormalities in patients with panic disorder led our unit to investigate the efficacy of carbamazepine in panic disorder patients. While ten of 14 completers demonstrated some improvement on carbamazepine, the overall clinical response was judged to be minimal as reflected by only a small decrement (-4.5 , $p < .02$) on the Zung Anxiety Scale and a nonsignificant change (-1.6 , $p = NS$) on the Spielberger State Anxiety Scale. Forty percent of the patients had a decrease in frequency of panic attacks on carbamazepine, while 50% had an increase and 10% showed no change. Neither the presence of EEG abnormalities nor prominent psychosensory symptoms predicted response to carbamazepine.

This is the first study, to our knowledge, to systematically examine the efficacy of carbamazepine in the treatment of panic disorder using a controlled, double-blind design. Our findings suggest that carbamazepine is of limited value in the treatment of most patients suffering from panic disorder with or without agoraphobia. On most outcome measures, carbamazepine failed to show any benefit over the preceding time period on placebo. While a small decrement was noted on the Zung Anxiety Scale, this was of minimal clinical significance.

d. Clonidine. In previous reports (Z01 MH 00071-06 BP), we presented the rationale for investigating the potential antianxiety effects following both an acute intravenous challenge (2 $\mu\text{g}/\text{kg}$) and chronic treatment with clonidine. The following summarizes our experience to date with this paradigm.

1. Acute Effects. Fourteen patients agreed to participate in the acute clonidine study. Of these 14, two did not complete both a placebo and a clonidine challenge. Only the 12 patients who completed both parts of the challenge (i.e., clonidine and placebo) are reported in the analysis. Seven women and five men made up this patient group. The mean age (\pm SD) was 36.2 ± 7.0 years (range 26-49 years).

Ten healthy subjects completed both the placebo and clonidine challenge. Nine women and one man made up this control group. The mean age (\pm SD) was 27.9 ± 13.6 years (range 19-56 years). The mean ages of the patient and control groups did not differ significantly (Student's t-test, $p = \text{NS}$). All patients were medication-free for at least three weeks prior to the start of the study.

Placebo failed to produce significant changes in state anxiety in the patients or normal controls ($p = \text{NS}$). While intravenous clonidine also failed to produce changes in state anxiety in the healthy subjects ($p = \text{NS}$), clonidine did produce significant decreases in Spielberger anxiety from a mean baseline value of 60.2 ± 11.5 to 47.1 ± 12.9 sixty minutes after clonidine (paired $t = 8.12$, $df = 11$, $p < .0001$) in the patients.

The placebo-corrected changes in anxiety with clonidine (i.e., change in anxiety with clonidine minus change in anxiety with placebo) were significantly greater in the patients than in the healthy subjects (-8.5 ± 8.5 versus -0.7 ± 6.0 , $t = -2.35$, $df = 19$, $p < .05$).

Healthy subjects showed a significant mean drop in systolic blood pressure with clonidine (-12.9 ± 7.3 mm Hg) as compared to placebo (0.7 ± 3.5 mm Hg, paired $t = -6.16$, $df = 9$, $p < .0005$). Patients also showed a significant mean drop in supine systolic blood pressure with clonidine (-14.6 ± 10.3 mm Hg) as compared to placebo (2.4 ± 9.7 mm Hg) (paired $t = -5.03$, $df = 6$, $p < .005$). Healthy subjects and patients did not, however, differ significantly in their supine systolic blood pressure response to clonidine ($t = -0.42$, $df = 16$, $p = \text{NS}$). The variance of the systolic blood pressure response to clonidine was not significantly different between healthy subjects and patients (Levene's F-test = 1.9, $p = \text{NS}$).

Neither group showed a significant change in diastolic blood pressure with placebo ($p = \text{NS}$). Healthy subjects showed a significant drop in supine diastolic blood pressure with clonidine (-8.9 ± 4.8 mm Hg, paired $t = -5.93$, $df = 9$, $p < .0005$), as did the patients (-10.4 ± 5.2 mm Hg, paired $t = -5.60$, $df = 7$, p

.001), but the two groups did not differ significantly in this response ($t = -0.63$, $df = 16$, $p = NS$). The variance of the diastolic blood pressure response to clonidine was not significantly different between healthy subjects and patients (Levene's F -test = 0.2, $p = NS$).

Neither group showed a significant change in heart rate with placebo ($p = NS$). Healthy subjects showed a significant drop in supine heart rate with clonidine (-6.7 ± 6.1 BPM, paired $t = -3.48$, $df = 9$, $p < .01$), with a similar trend seen for the patients (-4.0 ± 5.8 BPM, paired $t = -1.95$, $df = 7$, $p < .10$). Healthy subjects and patients did not differ significantly in their supine heart rate response to clonidine ($t = 0.95$, $df = 16$, $p = NS$). The variance of the heart rate response to clonidine was not significantly different between healthy subjects and patients (Levene's F -test = 0.1, $p = NS$).

There were no significant correlations between changes in anxiety and changes in systolic blood pressure or diastolic blood pressure. Decreases in anxiety with clonidine were not significantly correlated with increases in sleepiness in the healthy subjects ($r = .04$, $df = 6$, $p = NS$) or in the panic disorder patients ($r = .30$, $df = 10$, $p = NS$). Changes in heart rate with clonidine also were not significantly correlated with changes in anxiety in healthy subjects ($r = .43$, $df = 8$, $p = NS$) nor in patients ($r = .40$, $df = 6$, $p = NS$).

2. Chronic Effects. Eighteen patients agreed to participate in the chronic study. Five men and 13 women, with a mean age of (\pm SD) 34 ± 8.6 years (range 23-58 years), participated in this trial.

Clonidine failed to exhibit anxiolytic effects on any of the rating scales. All comparisons were made between pretreatment scores (while on placebo) and scores during the last week of treatment at the highest daily dosage administered. No statistically significant changes were noted on the Zung Anxiety Scale (-1.5 ± 10.3 , $p = NS$), Global Rating of Anxiety (-1.0 ± 2.0 , $p = NS$), or the HSCL-90 global severity index (-0.2 ± 0.5 , $p = NS$), anxiety subscale (-0.3 ± 0.9 , $p = NS$), phobic subscale (-0.3 ± 0.7 , $p = NS$), or panic subscale (-0.3 ± 0.8 , $p = NS$). A trend toward a decrement in anxiety was noted on the Spielberger State Anxiety (-4.4 ± 10.3 , $p < .10$), but the magnitude of this change was of minimal clinical significance.

The lack of apparent efficacy for the group as a whole notwithstanding, it is noteworthy that two patients demonstrated remarkable responses to treatment, showing a drop in Spielberger State Anxiety score of 22.7 and 21.3 points, respectively. The first patient, a 25-year-old woman, has not responded to any other treatment agent to date, including blind trials of imipramine, phenelzine, and carbamazepine. Moreover, the primary clinicians treating these patients rated ten of 14 patients as demonstrating some clinical benefit from clonidine pharmacotherapy. Of interest, the area of symptomatic improvement was inconsistent across patients. Thus, some patients demonstrated a marked reduction in panic attacks, but not generalized anxiety or agoraphobia. Other patients had a clinically significant improvement in generalized anxiety but experienced no blockade of panic attacks. As a result, a greater number of individual patients received clinical benefits from chronic clonidine pharmacotherapy than is readily evident from statistical analysis of separate rating scales.

D. Major Findings (Animal Research)

During the past three years, the Unit on Anxiety and Affective Disorders has established a viable colony of "normal" and "nervous" pure-bred pointer dogs. These dogs offer the advantage of investigating both "normal" behavior and "spontaneously-occurring" (rather than laboratory-conditioned) fear behaviors. The "nervous" line may be particularly useful in the study of several behaviors and characteristics relevant to human psychopathology, including genetically-transmitted inheritance with phenotypic expression of "nervous" behaviors at eight to 12 months of age. This delayed manifestation of pathology in dogs parallels in a similar, temporal fashion, the emergence of agoraphobia in humans during adolescence and early adulthood.

While the colony was being established the development of observation chambers with one-way mirrors and rating scales were developed. Careful and precise techniques for the surgical removal of the whole brain under general anesthesia were also developed and can be reliably performed under suitable conditions. A brain mold for the purebred pointer dog has been developed which allows the Unit to prepare regional brain tissue immediately after surgical removal for later measurement of transmitter levels and ligand binding to various receptors such as α_2 -adrenergic, benzodiazepine, imipramine, opiate, and other binding studies.

Research with this animal model has been conducted in collaboration with Drs. E. Klein, S. Steinberg, and S. Weiss. The following sections (D1-D4) report on preliminary findings with this model.

1. Heritability of Fear Behaviors

During the past year, the Unit has systematically evaluated the validity of the inheritance of nervous behaviors in the A- and E-lines of the Arkansas pure-bred pointer dogs. After breeding dogs from each line at our own facilities, the Unit blindly assessed the behaviors at nine months of age or older. We evaluated "nervous" behaviors using previously validated scales of fear or fear-related behaviors: weighted activity (WA), weighted nervous score (WNS), and new morbidity score (NMS).

The Unit developed several additional scores of fear based on 21 behaviors (i.e., tremor, circling, salivate, etc.) observed under four conditions (dog alone, dog exposed to human sitting on chair, human calling dog, human approaching dog).

On all measures, the offspring of E ("nervous")-line parents had significantly higher nervous scores compared to the offspring of A ("normal")-line parents. There were no sex differences in relation to fear or fear-related behaviors. These data confirm and extend previous observations demonstrating the heritability of fear behaviors in "nervous" pure-bred pointers.

2. Hearing and Non-hearing Pointer Dogs

During the past year, our unit was impressed by a behavioral pattern which was not mentioned in earlier work; namely, that nervous dogs seemed to be less responsive to the presence of a human if the human was not within their field of vision, suggesting a possibility of a hearing deficit. Such a deficit could potentially contribute to or largely determine the aforementioned abnormal behavior in the nervous dogs. Since a hearing deficit has not been previously described in these dogs, we decided to evaluate the hearing status of both nervous and normal dogs in our colony and further assess the relationship between a possible hearing deficit and the abnormal behavior.

The standard brainstem auditory-evoked response (BAER), which has been recently applied to dogs, was used for the assessment of hearing status. Of 16 normal dogs tested, all but one showed normal responses in both ears. One dog (a 4-year-old male) showed a normal response in one ear but no response in the other ear. Direct otoscopic examination gave no clue to the cause of this unilateral deficit. In contrast, the testing in the "nervous" dogs revealed that 20 of 27 dogs had no brainstem evoked response that could be detected in either ear. These dogs were thus considered to be deaf, while the remaining seven dogs had normal responses. However, behavioral ratings revealed that hearing and deaf dogs did not differ in their pathological response to the characteristic fear-provoking stimuli (e.g., human interaction), whereas both hearing and deaf nervous dogs markedly differed from normal dogs on this measure. Thus, regardless of the hearing status, there was a robust difference between nervous and control dogs. That is, these results support a conclusion that hearing status does not effect the behavioral outcome in these dogs and that these traits are not causatively related, although genetic linkage between the behavioral abnormality and the deafness might be expected.

3. Response to Diazepam and RO 15-1788

The Unit has investigated the effects of diazepam, RO 15-1788, and placebo in ten "nervous" and seven "normal" pointer dogs. Although RO 15-1788 reversed diazepam-induced hind leg ataxia in both lines, there were no significant drug group effects by ANOVA on the previously validated WA, WNS, NMS scales or the ten NIMH subscales of fear behaviors. These data suggest that most abnormal behaviors in this animal model of "anxiety" are unlikely to be mediated by an endogenously-produced, central type, benzodiazepine anxiogenic ligand. Also, the limited effects of diazepam somewhat parallel the relatively poor effects of this agent in the treatment of panic and agoraphobic syndromes in humans.

4. Yohimbine Binding

We studied α_2 -adrenergic receptor binding as determined by [3 H]-yohimbine in platelets and brains of the nervous and normal dogs in our colony. Our findings indicate that α_2 -adrenergic receptor density and affinity are similar in platelets and frontal cortex, but we did not observe significant differences in binding between the two groups of dogs.

5. Adenosine Binding

Since benzodiazepines and adenosine derivatives have marked effects on behavioral arousal, these systems were studied in the brains of both nervous and normal dogs. Adenosine receptors were found to be increased in the hippocampus, and adenosine reuptake sites were found to be increased in the cerebellum of the nervous dogs. No changes were observed in benzodiazepine binding. These findings suggest that adenosine neuromodulatory function might be impaired in the nervous pointer dogs. The report of increased sensitivity to caffeine in panic disorder patients compared to normal controls (see 11b and 11d), and the findings here of increased adenosine binding in the hippocampus in the nervous pointer dogs, provide indirect evidence for altered adenosinergic modulation in stress and anxiety syndromes.

6. Motor Activity

Nervous pointer dogs are characterized by a typical and highly reproducible pattern of fear-related behaviors which become manifest at the age of 3-9 months. These fear-related behaviors are most pronounced when these dogs are exposed to humans or novel environments. Upon such exposure these dogs frequently respond with reduced exploratory activity, hyperstartle, marked avoidance of the human observer, frequent catatonic freezing, cardiovascular changes, urination and defecation. In contrast, normal dogs do not demonstrate these abnormal behaviors under similar conditions. The marked motoric components of the fear response in the nervous dogs (reduced exploratory activity and catatonic freezing) prompted us to investigate spontaneous motor activity in these dogs to determine whether differences in motor activity between the two lines can be demonstrated under more "naturalistic", nonstressful conditions over a 24-hour period of time. Our unit studied spontaneous motor activity, using nontelemetric activity monitors, in both nervous and normal pointer dogs.

Data from eight normal dogs (two males and six females, mean age 10.5 ± 1 month) and ten nervous dogs (five males and five females, mean age 11.2 ± 2.2 months) were obtained. There was no significant difference in motor activity between the nervous and normal pointer dogs. Of interest, however, was the observation that the rest-activity cycle was not evident in either dog line.

II. Proposed Course

Efforts will be expanded to document the phenomenology, natural course, family dynamics, and personality structure of patients with panic disorder. Dr. T. Mellman will initiate a study exploring the impact of personality structure on the phenomenology and treatment of panic disorder. In collaboration with B. Scupi, a Panic Disorder Questionnaire, assessing current and past life experiences, has been developed. This Panic Disorder Questionnaire plus a number of standardized scales including the Parental Bonding Instrument, Retrospective Childhood and Current Fear Scale, Locke-Wallace Marital Satisfaction Inventory, Family Assessment Device, and the Childhood and Adult History Questionnaire will be administered. This study will assess whether retrospective perspectives of early childhood experience and family structure are associated with specific types of symptomatology or predict treatment outcome measures. In collaboration with Dr. J. Maser and B. Scupi, an inventory to assess phenomenology and natural course

of panic and the prevalence of co-morbidity of panic disorder with other psychiatric illnesses has been designed and will be administered to a large sample (> 2000) of subjects worldwide.

Human research conducted by the Unit on Anxiety and Affective Disorders has demonstrated an alteration in neuroendocrine and noradrenergic function in panic disorder patients compared to age- and sex-matched controls. These abnormalities include blunted GH, TSH, and ACTH to clonidine, TRH, and CRH, respectively. Disturbances in noradrenergic function are also suggested by increased DHE binding to platelets and increased sensitivity to the alpha₂-adrenergic antagonist, yohimbine. Other lines of evidence reviewed in previous reports (Z01 MH 00071-04/05/06 BP) also suggest a similarity in neuroendocrine and noradrenergic dysfunction in panic and major depressive disorders. As a result of these findings, the Unit will continue to investigate neuroendocrine and noradrenergic systems across a spectrum of mood and anxiety disorders.

We also intend to expand our research with caffeine. Further delineation of the clinical response to caffeine is indicated because caffeine consumption is correlated with symptoms of generalized anxiety in patients with panic attacks, but not in normal volunteers. Caffeine derivatives also activate noradrenergic activity in animals when iontophoretically applied to the locus coeruleus. Furthermore, caffeine has been shown to antagonize the biochemical and pharmacological effects of benzodiazepines, and alprazolam, a triazolabenzodiazepine, blocks the typical time course of caffeine-induced arousal, panic attacks, and generalized anxiety in humans (see Section C9). Other lines of evidence suggest a major role for adenosine-regulated systems in the mediation of caffeine's psychostimulant properties. All three of these systems have been independently implicated in the neurobiology of anxiety and stress. Moreover, caffeine was found by our Unit to significantly elevate measures of the stress-related hormone, cortisol, and induce cortisol escape from dexamethasone suppression. Thus, we intend to extend and expand our ongoing research with caffeine by investigating the behavioral, physiological, neuroendocrine, and biochemical effects of this and other methylxanthines in both animals and humans.

Drug trials with carbamazepine, clonidine, and verapamil have been concluded during the past year. While preliminary data suggest that each of these agents has significant antianxiety effects in a subgroup of panic disorder patients, none appears to demonstrate the same potency as standard antipanic agents such as imipramine and alprazolam. A new study investigating the effects of dipyridamole, compared to placebo and imipramine, will be initiated under the direction of Dr. M. Stein. The study of dipyridamole, an agent which blocks the reuptake of adenosine, reflects the Unit's interest in the role of adenosine systems in the neurobiology of anxiety disorders.

In addition to these drug trials in panic disorder, the Unit will expand its clinical studies to include patients with social phobias and obsessive-compulsive disorder. Since no controlled studies of the drug responsiveness of a pure social phobic sample have been completed, the value of pharmacological interventions with this disorder is unknown. A number of clinical studies have suggested that cognitive restructuring and exposure interventions are effective in the treatment of social phobia. During the next year, the Unit will complete a study to investigate the relative efficacy of alprazolam, phenelzine, and

cognitive-behavioral treatment of social phobia. This study will be conducted in collaboration with C. Shea, a doctoral candidate in clinical psychology at Catholic University. The inclusion of social phobics and obsessive-compulsive patients in the anxiety clinic will also provide a patient resource for investigating the specificity of several biological "markers" of panic disorder compared to patients with other severe forms of anxiety.

In the pointer dog model of "anxiety", the Unit will focus on the behavioral pharmacology of adenosine and adenosine derivatives and xanthine compounds. These experiments in animals will parallel our studies in humans investigating the neurobiology of fear-related behaviors and the mechanisms of antianxiety drugs.

Drug trials with novel antianxiety agents in humans and in "nervous" pointer dogs, in conjunction with concomitant measurements of their neurotransmitter effects, should enhance our understanding of alterations in neuroendocrine and neurotransmitter pathways associated with pathological human anxiety and animal fear, and lead to the development of more potent and specific pharmacotherapies.

III. Significance to Biomedical Research and the Program of the Institute

Several epidemiological surveys have suggested that pathological degrees of anxiety may adversely influence a large segment of our population. Panic disorder, an anxiety syndrome associated with agoraphobia, results each year in the impairment of individuals previously well-functioning and productive. Pathological anxiety has been recently found to be one of the most prevalent mental health problems in this country. The role of anxiety and stress in coronary heart disease and other medical illnesses has been suggested by a number of studies. Moreover, emerging epidemiological and familial data suggest that a subgroup of patients with major depressive illness plus panic attacks may represent an important and distinct subtype of major affective illness. We intend to investigate biological correlates in the plasma and cerebrospinal fluid of this subtype, who may be a greater risk for alcoholism and suicide, compared to patients with major depressive illness without panic attacks. An improved understanding of the clinical and biological aspects of both normal and pathological anxiety is thus critically needed. It is hoped that the developing battery of clinical and biological tests in patients with anxiety and related mood disorders will ultimately provide a clinical and biological profile of these illnesses and lead to more refined subcategorizations, as well as to more selective and efficacious treatment approaches.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00452-12 BP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuroendocrine Studies of Major Psychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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BPB, CNB, LCS, LNP, NIMH; DEB, EB, NICHD; SNB, NINCDS; SOB, NCI; LCS, NIAAA;
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LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Clinical Neuroendocrinology

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20892

TOTAL MAN-YEARS:

11.0

PROFESSIONAL:

12.0

OTHER:

4.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A primary focus of our work has been on mechanisms of physical and emotional stress and their relevance to major psychiatric and endocrine disorders. Our recent work with corticotropin releasing hormone (CRH) illustrates our comprehensive approach to this area of inquiry. For instance, we have advanced data which indicate that CRH is of physiological relevance to human pituitary-adrenal adrenal function, demonstrated its role in the pathophysiology of hypercortisolism in depression and anorexia nervosa, and administered it as a clinically useful means of determining the differential diagnosis between depression and Cushing's disease. Our data regarding interactions between the CRH system and the locus ceruleus-norepinephrine system suggest that a positive feedback loop between these two major effectors of the stress response may account for many of the clinical and biochemical manifestations of melancholic depression. As indirect support for a role of CRH in the mood component of depression, we have shown that procaine produces dose-dependent activation of pituitary-adrenal function in association with mood changes in patients with affective illness. Moreover, in vitro studies show procaine-induced dose-dependent activation of the CRH neuron which is prevented by carbamazepine. In clinical studies with volunteers and patients with panic disorder, we have implicated CRH in exercise and lactate-induced panic and have advanced in vivo and in vitro data that alprazolam may exert therapeutic effects by suppressing the CRH neuron. In studies exploring the mechanisms by which the immune system may stimulate adrenal corticosteroid counterregulation of the immune response, we have shown that both interleukin-I and interleukin-II produce dose-dependent activation of the CRH neuron.

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Project Description

A. Objectives

The fundamental strategy of the Section on Clinical Neuroendocrinology has been to extend current concepts regarding the physiology of neuroendocrine regulation in animal models and healthy volunteers, and to apply this understanding in an effort to unravel the pathophysiology of neuroendocrine disturbances in a variety of patient populations. Moreover, because neuroendocrine abnormalities constitute the cardinal manifestations of major psychiatric illness, such as depression and anorexia nervosa (vida infra), it is hoped that this work may unravel fundamental mechanisms of disease. Our clinical populations span multiple disciplines including patients with several major psychiatric diagnoses (e.g., affective illness, anorexia nervosa, panic anxiety disorder, schizophrenia, alcoholism), as well as a range of major neuroendocrine abnormalities (e.g., Cushing's disease, ectopic ACTH secretion, ACTH-independent hypercortisolism, primary and secondary adrenal insufficiency Nelson's syndrome, and diabetes insipidus).

Our work on neuroendocrine regulation in patients with major psychiatric illness reflects a rapidly enlarging body of literature which suggests an intimate linkage between neurohormonal functional activity and major components of the symptom complexes of illnesses such as depression and anorexia nervosa. For instance, several aspects of the syndromes of primary affective disorder and anorexia nervosa suggest hypothalamic dysfunction. Thus, patients with depression often manifest disturbances in appetite, libido, reproductive function (i.e., amenorrhea), water metabolism, cortisol secretion, and in the temporal organization of a variety of phenomena whose circadian periodicity is thought to be governed by hypothalamic pacemakers. Patients with anorexia nervosa show not only profound alterations in eating behavior, but also marked changes in hypothalamic-pituitary regulation, gonadotropin secretion, and in plasma levels of cortisol. In addition to efforts to explore the mechanism of these abnormalities in psychiatric populations, our interest in neuroendocrine systems also relates to the fact that the monoaminergic neurotransmitters long thought to play a dominant role in major psychiatric illness modulate the synthesis and release of a number of hypothalamic peptides and pituitary hormones; thus, examination of pituitary hormones in plasma can shed light on the functional activity of central biogenic amine systems. Moreover, the hypothalamic hormones themselves have been shown to be widely distributed within brain, to exert specific receptor-mediated biological actions, and to influence the functional activity of brain neurotransmitter systems. Several hypothalamic hormones have also been shown to have profound effects on coordinating complex behaviors and physiological processes of relevance to adaptation and the maintenance of internal homeostasis.

Our interest in neuroendocrine regulation in patients with medical illnesses such as Cushing's disease reflects our commitment to the clinical study of neuroendocrine regulation relevant to health and disease. We have focused our work on patients with abnormalities of the hypothalamic-pituitary-adrenal (HPA) function, because it is this axis which is most consistently disturbed in a variety of psychiatric illnesses; moreover, the hypercortisolism of depression can be of sufficient magnitude that it has been called a "pseudo-Cushing's state and can be difficult or impossible to distinguish from mild or

early Cushing's disease. In this regard, one of our goals was to comprehensively compare and contrast the pathophysiology of the hypercortisolism in depression and Cushing's disease, and to develop a means of assisting in their often difficult diagnosis. We also hoped to explore possible mechanisms for elucidating the differential diagnosis between Cushing's disease and ectopic ACTH secretion, and among various causes of adrenal insufficiency. Although our work in both psychiatric and medical populations focused on HPA regulation, we have also attempted to integrate data concerning the regulation of other neurohormones in these patients, including arginine vasopressin, oxytocin, somatostatin, the endogenous opiates, and growth hormone-releasing hormone.

In our clinical studies, several strategies have been routinely utilized: 1) direct measurement in the CSF and in the plasma of behaviorally active peptides during the basal state and/or following stimulation according to verified stimulation paradigms; 2) administration of hypothalamic releasing factors to test responses of the pituitary axis and to elucidate patterns of endogenous monoaminergic disturbance and neuroendocrine dysfunction; 3) elucidation of the effect of psychoactive agents on hypothalamic-pituitary axis function and on levels of behaviorally active peptides; 4) assessment of the temporal organization of neuroendocrine function; 5) administration of hormone antagonists to explore the functional relevance of various agents to normal physiology and to pathophysiological processes; 6) screening for restriction-fragment length polymorphism utilizing probes for genes whose functional regulation may be abnormal, based on our studies of neuropeptide dysregulation in major psychiatric illness.

As an adjunct to our clinical studies, we maintain an active pre-clinical studies program whose principal interests include the mechanisms of physical and emotional stress and the endocrine functions of the brain. In this regard, our pre-clinical program is closely allied with our clinical studies program, which we anticipate will focus on a coordinated series of studies to compare and contrast pathophysiological features in depression and anorexia nervosa. In this regard, both depression and anorexia nervosa can be precipitated by stress, and each is clearly influenced by the principal biologic effectors of the stress response. Moreover, neuroendocrine abnormalities constitute the cardinal biochemical manifestations of both depression and anorexia nervosa, and each constitutes the kind of syndromal process likely to be linked to alterations in one or more neurohormones known to function as integrative neuromodulators.

The specific pre-clinical components of our group which currently interface with our clinical program include studies utilizing immunocytochemistry, high resolution autoradiography, molecular biology (including in situ hybridization), and behavioral pharmacology.

Studies of Neuropeptide Regulation in Health and Disease:

- A. Studies of relevance in the comparison of pathophysiological mechanisms in depression and anorexia nervosa:

1. Corticotropin Releasing Hormone (CRH)

The stress responsiveness of anorexia nervosa and depression and the sustained hypercortisolism which is a consistent concomitant of these illnesses made the discovery of corticotropin releasing hormone an exciting event for endocrinologists and psychiatrists. Shortly after the sequencing of CRH, our group commenced a series of studies in healthy volunteers which validated the physiologic relevance of CRH to normal human pituitary-adrenal function, led to the development of strategies for studying pulsatile ACTH secretion, elucidated the biological and pharmacokinetic properties of ovine and human CRH, and demonstrated a circadian rhythm in the corticotroph's response to CRH and its dependence upon its exposure to pulsatile CRH for normal functioning.

Our studies of the normal physiology of the HPA axis have proven helpful in the establishment of a clinically applicable ovine corticotropin releasing hormone stimulation test which we have applied in a systematic way to tease apart pathophysiological mechanisms in psychiatric and neuroendocrine diseases. In patients with anorexia nervosa and depression, we showed that the response of the corticotroph cell to exogenous synthetic CRH was appropriately restrained by glucocorticoid negative feedback, indicating that hypercortisolism in these disorders reflected an abnormality at or above the hypothalamus, resulting in the hypersecretion of endogenous CRH. In support of this hypothesis was our data that healthy volunteers responded to a continuous infusion of CRH with a pattern and magnitude of cortisol secretion similar to that seen in anorexia nervosa and depression. Moreover, CSF CRH was frankly elevated in anorexia nervosa, and correlated positively with post-dexamethasone cortisol levels in depression.

In addition to our elucidation of the mechanism of hypercortisolism in depression and anorexia nervosa, we also hypothesized that CRH may play an important role in the overall symptom complexes of these two illnesses. Thus, the ICV administration of CRH to experimental animals produced many of the physiological and behavioral concomitants of these disorders, including not only hypercortisolism but also hypothalamic hypogonadism, decreased libido, anorexia and increased motor activity.

Although we have no definitive information regarding the cause of the dysregulation in the CRH system in anorexia nervosa and depression, our in vitro studies show that norepinephrine and serotonin are potent stimuli to the CRH neuron, while GABA is inhibitory. Moreover, we noted a positive correlation between CSF CRH and CSF NE in depression, suggesting that activation of the locus ceruleus-norepinephrine (LC-NE) system may play a particularly important role in the dysregulation of CRH in this disorder. In this regard, the recent report that CRH markedly increases the firing rate of the LC-NE system suggests that a positive reverberatory feedback loop between the CRH and the LC-NE system (e.g., the two major effectors of the stress response) may account for many of the clinical and biochemical manifestations of depression. In contrast to depression, Kaye et al. have shown that indices of NE-LC system function are generally diminished in patients with anorexia nervosa, regardless of clinical state.

2. Peripheral and Central Vasopressin Regulation

In studies of vasopressin regulation in anorexia nervosa, we have shown that underweight subjects exhibit a previously undescribed abnormality in which plasma vasopressin secretion proceeds entirely dissociated from osmotic control. This defect in plasma vasopressin secretion was manifested clinically as an intermittent inappropriate vasopressin secretion syndrome, which could contribute to hemodilution and to the life-threatening hypokalemia seen in underweight anorexic subjects. An additional finding was that the abnormal osmoregulation of plasma vasopressin secretion was almost always associated with indices of the hypersecretion of centrally directed vasopressin into the CSF, a defect which persisted not only in patients re-studied following the short-term correction of the weight loss, but in many after the long-term correction of weight loss as well. We have previously postulated that the increase in centrally directed vasopressin secretion might influence the symptom complex of anorexia nervosa. Hence, extensive experimental evidence shows that not only does AVP potentiate the actions of CRH, but that AVP also delays the extinction of learned behaviors acquired during aversive conditioning - an effect analogous to the anorexic person's perseverative preoccupation with the adverse consequences of eating. Synergy between AVP and CRH could also contribute to the substantially higher magnitude of hypercortisolism in anorexia nervosa.

In contrast to patients with anorexia nervosa, patients with depression showed intact osmoregulation of plasma vasopressin secretion, though the quantitative vasopressin response to osmotic stimulation (i.e., the sensitivity of the osmoreceptor) is significantly reduced in depression. This reduction in osmotically mediated vasopressin secretion was associated with a significant decrease in the secretion of vasopressin into the CSF of depressed patients. Thus, as with indices of LC-NE function, the functional activity of centrally directed vasopressin seems abnormal in opposite directions in depression and anorexia nervosa.

3. Growth Hormone Regulation

Integrated basal growth hormone secretion is increased in both depression and anorexia nervosa. To explore the mechanism of the hypersecretion of growth hormone in these disorders, we administered synthetic growth hormone releasing hormone (GH-RH 1-44) to drug-free anorexic and depressed patients. Surprisingly, the GH responses in these populations were in the opposite direction. Hence, in patients with anorexia nervosa, GH responses to GH-RH were exaggerated at reduced weight and returned to normal after the short-term correction of the weight loss, while in depression the GH responses to GH-RH were blunted, returning to normal after clinical recovery. Our data indicate that in the underweight anorexics, both the more severe hypercortisolism and the weight loss per se result in a decrease in somatomedin C production and/or biologic efficacy, so that the reduced restraint of somatomedin C upon the somatotroph allows an exaggerated GH response to GH-RH. In depression, on the other hand, the hypercortisolism is not of a sufficient magnitude to inhibit somatomedin C production and/or actions, but of subsequent magnitude to inhibit the GH response to GH-RH at the level of the pituitary corticotroph cell.

B. Additional Studies of CRH Regulation of Potential Relevance to Depression: In vivo and in vitro Studies of Procaine Effects on Mood and Neuroendocrine Function

Anatomic and physiologic considerations suggest that alterations in the functional activity of certain limbic structures, including amygdala and hippocampus, may play a role in both the symptom complex and neuroendocrine disturbances seen in affective disorders. Hence, direct stimulation of these structures in humans has been shown to produce sensory experiences with often vivid emotional recall, and to reproduce many of the mood states characteristic of clinical depression. Moreover, studies utilizing electrical stimulation of limbic structures, or their spontaneous activation through seizure activity, suggest that these brain areas can exert a potent influence on the secretion of stress-associated hormones. Because the use of these methods in clinical studies of depression is severely limited by ethical considerations, we felt that availability of a safe pharmacologic probe of limbic activity would greatly enhance our understanding of the role of these structures in the pathophysiology of affective disturbances.

A number of experimental observations suggested that local anesthetics (e.g., procaine, lidocaine, cocaine) might be candidates for such a pharmacologic probe of limbic activity. Hence, local anesthetics given chronically to rats can produce kindled seizures which cross-sensitize with amygdala-kindled seizures and which are associated with marked increases in metabolic activity in specific limbic areas, including amygdala and hippocampus. Moreover, previous studies had suggested that intravenous procaine administration in humans could produce many of the same psychosensory and mood effects seen with electrical stimulation of limbic structures. We were particularly interested in the potential involvement of CRH in these effects, as we have shown that intracerebroventricular CRH can produce amygdaloid seizures in rats which sensitize animals to the development of lidocaine-kindled seizures.

To explore the potential relationship between CRH and limbic seizures of potential relevance to affective disorder, we gave IV procaine to healthy volunteers, patients with affective disorder (who tend to have sustained changes in mood) and patients with borderline personality disorder (who tend to have paroxysmal mood shifts which have been previously postulated to reflect limbic system instability). We have previously shown that procaine is capable of inducing psychosensory and EEG effects suggestive of action at temporal lobe and limbic structures. Procaine stimulated ACTH, cortisol, and prolactin, but not growth hormone secretion in our subjects in all diagnostic groups. Although the magnitude of the ACTH responses were similar, there was significantly greater variability of the response in the borderline group compared to controls. As yet, the group of affective disorder patients is too heterogeneous with respect to state to allow meaningful comparisons.

We have attempted to clarify the mechanism of the ACTH response to procaine using an *in vitro* short-term rat hypothalamic organ culture system (1 hypothalamus per well) in which we have previously demonstrated robust CRH responses to predicted provocative stimuli. In this system, procaine, lidocaine, and cocaine all stimulated CRH release in a dose-dependent fashion in the micromolar concentration range; these concentrations are attainable in the plasma after IV administration to humans at the dose range used in our clinical

cal studies.

In a subsequent study exploring the effects of carbamazepine procaine-induced in vitro CRH secretion, we showed that this anti-seizure, anti-kindling agent significantly reduced the CRH response to incubation with procaine. Hypothetically, we postulate that carbamazepine may exert anti-kindling and mood-stabilizing effects via inhibition of the effect of stimuli which activate the CRH neuron. These findings suggest that local anesthetics are capable of eliciting many of the behavioral and neuroendocrine alterations seen in depression, and that some of these effects may be mediated by activation of limbic-hypothalamic pathways, resulting in release of CRH. Moreover, the therapeutic effects of carbamazepine may confer protection against excessive CRH release by pathogenic stimuli.

C. The Potential Role of CRH in the Pathophysiology of Panic Disorder: In Vivo and In Vitro Findings

When administered via the intraventricular route to experimental animals in low to moderate doses, CRH initiates a coordinated series of physiological and behavioral responses similar to those seen during adaptation to stressful situations; these include not only pituitary-adrenal activation, but also activation of the sympathetic nervous system, decreased feeding and sexual behavior, arousal, enhanced learning and memory, and increased aggression. Like many other neuropeptides which produce arousal, the central effects of CRH seem to follow an inverted U-shaped dose response curve, so that at higher doses maladaptive, "anxiogenic" effects are produced. These include decreased exploration in an open field, enhanced responsiveness to acoustic startle, disruption in learning and memory, and immobility. These behavioral effects in experimental animals stimulated us to explore the potential role of CRH in the panic disorder syndrome.

In a series of clinical and pre-clinical studies, we advanced the following lines of evidence suggesting a role for CRH in panic disorder: 1) Patients with panic disorder, who often manifest basal hypercortisolism, show an attenuated ACTH response to ovine CRH (oCRH) analogous to that described in our patients with depression and anorexia nervosa. 2) Exercise, which can precipitate panic attacks in patients with panic disorder, produces dose-dependent increases in plasma ACTH and cortisol secretion in volunteers (when applied in graduated levels of 50%, 70%, and 90% of the maximal oxygen utilization rate). As a corollary, this exercise-induced dose-dependent ACTH secretion correlated positively with levels of plasma lactate, another stimulus of panic attacks. 3) Studies of the in vitro regulation of the rat hypothalamus show that lactate produces dose-dependent increases in CRH secretion. 4) Alprazolam, one of the most potent anti-panic agents, inhibits serotonin-induced CRH secretion in doses as low as 10^{-9} M and was substantially more potent than was diazepam, a less effective therapeutic agent. 5) Intravenous alprazolam given to non-restrained primates with indwelling vena cava lines inhibited ACTH and cortisol secretion in a dose-dependent fashion.

In summary, we have advanced several lines of circumstantial evidence suggesting the potential role of CRH in the pathophysiology of panic disorder. The potential involvement of CRH in the hypercortisolism in disorders such as depression, anorexia nervosa, and panic disorder is of interest in light of evi-

dence that the overall group of patients with these illnesses shows a greater than normal family history for primary affective disorder. This suggests that these disorders may lie along a spectrum of depressive disorders with CRH dysregulation as a common denominator.

D. Studies of the Differential Diagnosis and Pathophysiology of Depression and Cushing's Disease: Focus on CRH and Related Neuropeptide Systems

Depression is often the first sign of Cushing's disease, preceding the physical stigmata by months or years, while the depressed phase of primary affective disorder is often associated with profound hypercortisolism (often termed a pseudo-Cushing's state). Hence, the differential diagnosis between early or mild Cushing's disease and depression can be impossible to ascertain, and their clinical and biochemical similarities have suggested that they share a similar pathophysiological basis.

To explore patterns relating to the differential diagnosis and pathophysiology in depression and Cushing's disease, we utilized either the administration of CRH or measurement of CRH and related peptides in the cerebrospinal fluid (CSF). By these means, we hoped to develop a clinically useful means to distinguish these two entities and to determine if the two illnesses represent similar or distinct pathophysiological mechanisms.

Our first line of investigation was to test the functional integrity of the pituitary corticotroph cell in our patients following the administration of oCRH. Patients with Cushing's disease showed markedly exaggerated ACTH response to oCRH despite their profound basal hypercortisolism, indicating a gross defect in glucocorticoid negative feedback upon the pituitary corticotroph cell. This contrasts markedly with the attenuated ACTH responses to oCRH that our group has shown in depression, which indicate a pituitary corticotroph cell appropriately restrained by hypercortisolism. On the basis of these divergent responses to oCRH, which reflect different pathophysiological mechanisms, the oCRH test has proven helpful in the differential diagnosis between depression and Cushing's disease. This is so even when basal plasma cortisol values overlap, and is in marked contrast to all other noninvasive diagnostic procedures.

The differential pituitary corticotroph cell function in Cushing's disease and depression help explain the fundamental differences in the clinical manifestations of hypercortisolism in these disorders. For instance, the fact that ACTH secretion can proceed relatively unchecked by cortisol negative feedback in Cushing's disease allows for the kind of sustained hypercortisolism required to produce Cushingoid stigmata. On the other hand, although depressed patients can have cortisol levels in the Cushingoid range, their intact cortisol negative feedback upon the pituitary means that any centrally mediated rise in plasma ACTH and cortisol secretion would lead to a secondary glucocorticoid restraint of the pituitary, with a subsequent fall in the plasma cortisol. Hence, it is highly unlikely that the kind of sustained hypercortisolism required for Cushingoid features could supervene.

We also wished to determine whether the hypothalamic CRH neuron in Cushing's disease was hyperactive as in depression, or was restrained by long-

standing hypercortisolism. Three lines of evidence support the latter conclusion: 1) Twenty-two of 26 patients with Cushing's disease, whom we studied one week after transsphenoidal adenomectomy (at a time when basal ACTH levels were uniformly undetectable), nevertheless showed ACTH responses to endogenous CRH; from this we surmise that the post-operative adrenal insufficiency in the patients reflects suppression of CRH neurons by long-standing hypercortisolism; 2) Pituitary corticotroph cell function could be restored post-operatively by repeated priming doses of human CRH for several days (analogous to the situation in hypothalamic LH-RH deficiency in which spontaneous LH and FSH secretion could only be elicited in the context of priming doses of LH-RH); and 3) CSF CRH levels in Cushing's disease were significantly lower than in depressed patients or controls.

Because CSF somatostatin levels are significantly reduced in depression, we measured this peptide in the CSF of patients with Cushing's disease. Somatostatin levels in Cushing's disease were also significantly reduced, suggesting that hypercortisolism may be a factor in the decreased somatostatin levels in both Cushing's disease and depression. Though the functional consequences of this reduction in CSF somatostatin are unknown, it may not be coincidental that Cushing's disease joins depression and Alzheimer's disease as disorders characterized by diminished cognitive performance and decreases in central somatostatin levels.

E. Studies of the Circadian Organization of Pituitary-adrenal Function in Patients with Affective Disorder

In an extensive series of depressed patients and control subjects, we have assessed plasma ACTH and cortisol levels every 30 minutes for 40 hours (encompassing two nights and one day). We noted that although the mean 40-hour cortisol secretion was significantly greater in depressed patients than in controls, the mean ACTH concentration was similar. However, depressed patients consistently showed a greater number of ACTH and cortisol secretory episodes/24 hours than did controls, thus giving greater total ACTH as well as cortisol secretion than did controls. These data support our formulation that the adrenal has become hyperresponsive to ACTH in patients with depression and that the hypersecretion of CRH takes the form of increased pulsatile secretion as at least one component in the pathophysiology in HPA function in depressed patients. A potentially important methodologic point was the finding that in controls the pattern of ACTH and cortisol secretion was essentially identical on each of two successive nights. While there was no significant difference in these parameters on two successive nights of sampling in depressed patients, there was more variability in this group than in the control subjects.

F. Studies of ACTH Secretion into the CSF in Patients with Depression, Anorexia Nervosa, Cushing's Disease, and Alzheimer's Disease

The previous sections have reviewed our work with CRH and have included extensive data concerning the secretion of ACTH into plasma. We have also explored the secretion of ACTH into the CSF. Our data in stalk-sectioned primates, indicating substantial levels of ACTH in CSF despite barely detectable plasma ACTH levels, indicates that the pituitary may not be the source of CSF ACTH. Rather, the ACTH found in the CSF most likely is derived from the arcuate nucleus, which contains POMC and such post-translation products as ACTH, beta-lipotropin, and beta-endorphin.

In several separate populations of normal volunteers, we have consistently noted a significant positive correlation between CSF CRH and CSF ACTH. This finding is compatible with anatomic data that CRH cell bodies in the paraventricular nucleus send nerve terminals to the arcuate nucleus, and indicates that CRH effects upon central POMC may be analogous to its effects upon the POMC residing within the pituitary corticotroph cell.

Studies in patient populations show that CSF ACTH is markedly reduced in both patients with Cushing's disease and the ectopic ACTH syndrome. These data suggest that the secretion of ACTH into the CSF is glucocorticoid-suppressible. CSF ACTH levels also tend to be low in depressed patients, but the levels are not nearly as suppressed as in Cushing's disease. In fact, there is little overlap between the two groups, so that this measure may prove helpful in the differential diagnosis of depression and Cushing's disease. Of potentially even greater diagnostic significance is the plasma:CSF ratio of ACTH in depression and Cushing's disease. In normals and depressed patients, this ratio is almost always greater than 1.0. Conversely, in patients with Cushing's disease, the confluence of high basal plasma ACTH and low CSF ACTH values produces a plasma:CSF ACTH ratio which is almost always less than 1.0.

A marked reduction in ACTH and other POMC fragments was also found in underweight patients with anorexia nervosa (i.e., ACTH, beta-lipotropin, beta-endorphin, and N terminal fragment). The cause of these abnormalities is unknown, but may reflect the restraining influence of hypercortisolism or an effect of CRH neuron hyperactivity to deplete the pool of POMC fragments which is ordinarily secreted into the CSF. CSF ACTH levels return to normal upon the short-term correction of weight loss in anorexia nervosa, and are also normal in patients studied months to years after restoration of normal weight.

CSF ACTH was also significantly reduced in patients with Alzheimer's disease. On the other hand, their levels were normal in subjects with schizophrenia and in alcoholic patients studied at one and three weeks after abstinence from drinking.

The functional consequences of the apparent reduction of CSF ACTH or other POMC fragments in Alzheimer's disease or anorexia nervosa is not known. Data from studies in experimental animals suggest a possible role for ACTH in mediating sexual behavior. Additional studies in experimental animals also suggest that ACTH may enhance certain components of information processing.

Pre-clinical Studies:

A. Regulation of CRH Secretion

We have recently worked to develop a sensitive in vitro hypothalamic rat organ culture system to explore the regulation of the hypothalamic neuron. We have examined the effects of several substances, including serotonin, acetylcholine, epidermal growth factor, and norepinephrine.

Serotonin (5HT) stimulated immunoreactive (IR) corticotropin releasing hormone (CRH) secretion by rat hypothalamic explants after an overnight pre-incubation but not immediately after explantation. This response was mediated primarily by 5HT₂ receptors, as suggested by the complete inhibition of sero-

tonin-induced rat CRH (rCRH) secretion by ritanserin. Neither atropine and hexamethonium nor phentolamine inhibited 5HT-induced IR-rCRH secretion, suggesting that it is not mediated by cholinergic or alpha-adrenergic interneurons. In contrast to 5HT, KCl-induced depolarization increased IR-rCRH secretion both after overnight incubation and immediately after explantation. On both occasions the IR-rCRH content of hypothalami and their histological examination were similar. The bulk of IR-rCRH secreted from the hypothalamic explants under basal conditions and after KCl stimulation, coeluted with synthetic rCRH on Sephadex G-75 gel filtration chromatography.

Acetylcholine (ACH) also stimulated hypothalamic IR-rCRH secretion in a dose-dependent fashion, at concentrations ranging from 10^{-9} M to 10^{-5} M. This effect was antagonized by the simultaneous presence of atropine and hexamethonium, a muscarinic and a nicotinic receptor antagonist, respectively ($p < 0.05$). Further evidence for the cholinergic regulation of the CRH neuron was provided by the findings that both carbachol, a muscarinic receptor agonist, and nicotine, a nicotinic receptor agonist, stimulated IR-rCRH secretion in a dose-dependent fashion. These effects were antagonized by atropine and hexamethonium, respectively, suggesting that both muscarinic and nicotinic receptors are involved in the process. ACH also stimulated hypothalamic IR-rCRH secretion in the presence of phentolamine and kitanserin. Hence, we conclude that ACH stimulates hypothalamic CRH secretion via both muscarinic and nicotinic receptor mechanisms. This effect is not mediated by a serotonergic or alpha-adrenergic interneuron. These data suggest that acetylcholine may be implicated in the regulation and the stress activation of the HPA axis in vivo.

Epidermal growth factor (EGF), a polypeptide mitogen that participates in wound healing, has ACTH-like activity in ewes. We examined its effects on the primate hypothalamic-pituitary-adrenal (HPA) axis by administering EGF intravenously (0-100 ug/kg) to rhesus monkeys. EGF caused dose-dependent elevations of plasma ACTH and cortisol in these animals. To define whether the locus of stimulation was the hypothalamus and/or the pituitary gland, we examined the capacity of EGF to directly stimulate hypothalamic corticotropin releasing hormone (CRH) or pituitary ACTH secretion in a rat hypothalamic organ culture system and a rat pituitary cell system. EGF-stimulated hypothalamic CRH release in a dose-dependent manner, but failed to cause pituitary ACTH release. Hence, EGF, in addition to its previously described ACTH-like activity, also has CRH-releasing activity in the primate. In this regard, EGF may participate in the physiologic activation of the HPA axis at times during which EGF concentrations are raised. These include such states as trauma, surgery, and possibly emotional stress.

We explored the effect of norepinephrine on CRH secretion because of a growing body of data which indicate that the noradrenergic and CRH systems may interact to play a principal role in modulating the responses to physical and emotional stress. Moreover, an extensive series of previous studies have provided conflicting results regarding the effects of catecholamines on the central component regulating pituitary-adrenal function.

Norepinephrine (NE) stimulated IR-rCRH secretion in a dose-dependent fashion with peak effects in the nM range. The effect of NE was antagonized by the

alpha antagonist phentolamine, by the alpha₁ antagonist prazosin and by the alpha₂ antagonist yohimbine, but not by the beta blocker L-propranolol. Compatible with these data were the findings that the alpha₁ agonist phenylephrine and the alpha₂ agonist clonidine both stimulated IR-rCRH secretion in a dose-dependent fashion. On the other hand, while the beta agonist isoproterenol caused a weak, non-dose dependent increase in IR-rCRH secretion, this effect could not be antagonized by L-propranolol. Despite pre-treatment with antagonists to serotonin and acetylcholine receptors, the effect of NE upon IR-rCRH secretion was undiminished. On the other hand, pre-treatment with gamma-aminobutyric acid (GABA) significantly attenuated NE-induced IR-rCRH secretion.

Epinephrine-stimulated IR-rCRH secretion, but only at higher concentrations. This stimulatory effect was antagonized by phentolamine, but not by L-propranolol. Dopamine (DA) had a weak stimulatory effect that could be antagonized by the DA₁ receptor antagonist SCH 23390, but not by phentolamine.

We conclude that NE stimulates hypothalamic IR-rCRH secretion via hypothalamic alpha₁ and alpha₂ receptors. The effect of NE upon IR-rCRH secretion does not appear to be mediated by serotonergic or cholinergic interneurons, but is modulated by the inhibitory neurotransmitter GABA. The present data support the idea that NE is excitatory rather than inhibitory upon CRH secretion when it acts directly at a hypothalamic locus. These findings may be of clinical relevance to illnesses such as depression, which are characterized by activation of both central NE and CRH systems. Hence, activation of the central NE system may contribute to the hypercortisolism characteristic of this disorder.

B. Studies Utilizing an in vitro Perfusion System for Studying the Hormonal Functions of the Human Placenta:

Pregnancy is the only known state in which CRH circulates in plasma at levels expected to cause activation of the pituitary-adrenal axis (100 pg/ml up to 4,000 pg/ml). These levels gradually increase even further during labor. Plasma CRH concentrations return to undetectable (< 15 pg/ml) levels following delivery. Interestingly, it has been known for years that pregnancy is characterized by hypercortisolism to a degree similar to what has been observed in severe depression and anorexia nervosa.

To study the potential role of the placenta as a source of plasma CRH during pregnancy, we have developed an in vitro perfusion system in which full thickness placenta fragments are kept in culture. These fragments contain a 1300-nucleotide-long mRNA which hybridizes with a CRH specific cDNA probe. Glucocorticoids, prostaglandins, catecholamines, oxytocin and vasopressin have an inhibitory or stimulatory effect on placental secretion of CRH, which is chromatographically identical to hypothalamic CRH. Our perfused placenta fragments also secrete immunoreactive beta-endorphin. Both CRH and oxytocin at 10⁻⁸ M concentrations stimulate the secretion of beta-endorphin by the placenta. These findings have raised the following questions: 1) Does the high intra-pregnancy CRH suppress hypothalamic secretion of CRH (via cortisol negative feedback) and therefore lead to a brief post-partum adrenal insufficiency state, followed by a later rebound of endogenous hypothalamic CRH secretion 6-8 weeks later? Interestingly, the very common "post partum blues"

occur in the first week post partum whereas post partum depression occurs 1-3 months later. 2) What regulates the secretion of placental CRH? Mechanical contraction or ischemia of the placenta may be responsible for the elevations of plasma CRH seen during labor. 3) What is the function of pregnancy plasma CRH other than stimulating the maternal pituitary-adrenal axis? Do the vasodilatory properties of CRH play role in labor? For instance, CRH-induced superior mesenteric vessel dilation may protect the maternal intestinal tract from ischemia. Moreover, circulating CRH might regulate uterine blood vessel flow.

C. Studies with an in vivo Primate Model:

We have recently adapted a technique for surgical implantation of a vena cava cannula which allows continuous access to the intravenous space of non-restrained non-human primates. The purpose of our primate laboratory is to allow extensive study of the physiology of hypothalamic-pituitary-adrenal function and to explore our hypotheses regarding the pathophysiology of illnesses such as depression and anorexia nervosa. Findings to date include: 1) demonstration of dose-dependent effects of alprazolam in suppressing pituitary-adrenal function, compatible with our in vitro data showing that this agent is a potent suppressor of the CRH neuron; 2) demonstration of dose-dependent effects of procaine to stimulate the pituitary-adrenal axis, compatible with our in vitro data that procaine is a potent stimulus to the CRH neuron; 3) demonstration of a potent effect of epidermal growth factor (EGF) to stimulate pituitary-adrenal function in doses comparable to CRH itself, compatible with our in vitro data that this growth factor stimulates the CRH neuron and the data of others that EGF may antagonize glucocorticoid effects by phosphorylating (and hence inactivating) lipocortin, the lipoprotein thought to mediate many glucocorticoid effects; 4) establishment of dose-response relationships for vasopressin-induced ACTH secretion; 5) elucidation of the effects of adrenalectomy and high-dose RU 486 on pituitary adrenal function (e.g., time course of ACTH elevation to peak levels, effects on pulsatility, doses of glucocorticoids required to restrain the hypothalamic-pituitary components of the axis).

Significance to Biomedical Research and the Program of the Institute

The Section on Clinical Neuroendocrinology has attempted to extend current concepts regarding the physiology of neuroendocrine regulation in animal models and healthy volunteers, and to apply this understanding in an effort to unravel the pathophysiology of neuroendocrine disturbances in a variety of patient populations. Such clinical populations span multiple disciplines, including patients with major psychiatric and major neuroendocrine illnesses.

Our work with corticotropin releasing hormone serves to illustrate the implementation of our basic strategy for research. For instance, with respect to normal physiology, we advanced the first data that CRH was of physiologic relevance to the regulation of basal circadian pituitary-adrenal function in man. Our data in controls, however, also support the idea that stress-induced ACTH secretion requires factors other than CRH alone. These findings, as well as our finding that the pituitary corticotroph, like the gonadotroph, requires the priming effects of its hypothalamic releasing hormone, have added to the understanding of hypothalamic-pituitary-adrenal physiology in human subjects.

In further studies with volunteers, our group was the first to compare the

pharmacokinetic properties of ovine and human CRH. Moreover, on the basis of this data, we concluded correctly that oCRH was best suited for diagnostic tests while hCRH was most suited for studying pulsatile ACTH secretion. Dose-response studies, which revealed the lowest maximal stimulatory dose for ACTH and cortisol secretion, subsequently facilitated the clinical application of a CRH stimulation test. Our demonstration that hormonal responses to oCRH were greater in the evening than in the morning further allowed us to refine this CRH stimulation paradigm.

Our CRH stimulation paradigm has found widespread clinical application. Indeed, the results of our studies have clarified some of the most elusive and difficult clinical problems in endocrinology. For instance, the differential pituitary-adrenal responses to CRH in depression and Cushing's disease contributed to the conclusion that hypercortisolism in these illnesses reflects abnormalities at different loci in the adrenal axis. Moreover, the CRH stimulation test represents a substantial advance over any previous diagnostic test as an aid in determining the differential diagnosis between depression and early or mild Cushing's disease. The CRH stimulation test has also proved helpful in distinguishing Cushing's disease from ectopic ACTH secretion and in distinguishing subtypes of secondary adrenal insufficiency (i.e., those secondary to either selective corticotroph cell deficiency vs. corticotroph sparing or hypothalamic CRH deficiency).

Our conclusion that hypercortisolism in depression reflects hypersecretion of endogenous CRH is of interest, not only because it clarifies the pathophysiology of HPA dysfunction in depression, but also because CRH administration to experimental animals reproduces many of the components of the symptom complex of affective illness, such as hypothalamic hypogonadism, decreased libido, anorexia, irritability, and changes in motor activity. Moreover, CRH given ICV produces limbic seizures which cross-sensitize with electrically kindled seizures. Our hypothesis that CRH may constitute an important link between stressful early life experience and the syndrome of recurrent depression has become one that is being vigorously pursued in many major research centers.

Our pre-clinical data have helped us to further extend our hypothetical mode of depression to include potentially synergistic interactions between the CRH system and the locus ceruleus-norepinephrine (LC-NE) system. Hence, our in vitro hypothalamic organ culture studies have shown that norepinephrine is a potent stimulus to CRH secretion. This finding, in association with the finding that CRH markedly increases the LC firing rate, suggests that the CRH and LC-NE system (i.e., the two major effectors of the stress response) may participate in a mutually reinforcing reverberatory loop which may be responsible for many of the clinical and biochemical manifestations of depression. This hypothesis is fully elaborated in much greater detail in an invited paper solicited by The New England Journal of Medicine, entitled "Clinical and Biochemical Manifestations of Depression: Relationship to the Neurobiology of Stress."

Our studies have also significantly added to an understanding of pathophysiological mechanisms in anorexia nervosa. For instance, our data that hypercortisolism in anorexia nervosa reflects pathophysiological alterations

similar to those seen in depression have considerably strengthened the idea that depression and anorexia nervosa share important features in common and may reside together within a broad depressive spectrum disorder. This is especially so because, as noted, CRH administration to experimental animals produces many behavioral and physiological features common to both depression and anorexia nervosa.

Several lines of evidence advanced by our group have led to a testable hypothesis regarding the role of CRH in the pathophysiology of panic disorder. These data, reviewed in detail in this summary, suggest that CRH may be involved not only in the hypercortisolism of this disorder, but also in exercise and lactate-induced panic episodes. Moreover, we have shown in a series of *in vivo* and *in vitro* studies that alprazolam is a much more potent inhibitor of the pituitary-adrenal axis and the CRH neuron than diazepam, compatible with its clinical superiority in the treatment of panic disorder.

Our studies of vasopressin regulation in anorexia nervosa have also clarified the pathophysiology of disturbed water metabolism in these subjects. Specifically, we described a novel defect in plasma vasopressin secretion, in which this parameter had become entirely uncoupled from osmoreceptor control. Moreover, we noted that this defect in plasma vasopressin regulation was almost always associated with either hypersecretion of vasopressin into the CSF space or a reversal of the normal plasma CSF vasopressin gradient. The finding of increased central vasopressin secretion in anorexia nervosa is of potential interest in light of data that vasopressin delays extinction of learned behavior acquired during aversive conditioning, and the observation that a fundamental abnormality in anorexia nervosa is a perseverative and exaggerated sense of the adverse consequences of eating. Parenthetically, the findings of enhanced central secretion of CRH and vasopressin in anorexia nervosa may contribute to the particularly pernicious, treatment-resistant quality of this disorder, because these two peptides exert synergistic effects on the pituitary corticotroph cell, and may do so within the CNS.

Our work has also helped to clarify the pathophysiology of growth hormone hypersecretion in anorexia nervosa. Our findings, based on the first application of GH-RH to the study of anorexia nervosa, suggest that the hypersecretion of GH in this disorder may, in part, reflect a functional deficiency of somatomedin C, which exerts a tonic restraining effect on the pituitary somatotroph cell.

Our pre-clinical studies have helped to extend knowledge regarding the regulation of the CRH neuron. Hence, we determined the major stimulatory (norepinephrine, serotonin, acetylcholine) and inhibitory (GABA/benzodiazepine system) neurotransmitters influencing CRH secretion and demonstrated the presence of both short- and ultra-short negative feedback loops via ACTH and CRH, respectively. We have also shown that several substances secreted during physical stress, such as interleukin I, interleukin II, epidermal growth factor, and lactate may be CRH secretagogues of physiological relevance.

Future Directions

A. Comparative Studies of Depression and Anorexia Nervosa

One of our fundamental hypotheses is that depression and anorexia nervosa lie on a broad continuum of depressive spectrum disorders with CRH dysregulation as a potential link of relevance to many overlapping components of these illnesses (e.g., hypercortisolism, hypothalamic hypogonadism, decreased libido, anorexia, and increased motor activity). Our work in exploring the dysregulation in CRH function common to both illnesses will attempt to further document both the presence and the pattern of this defect and to explore its possible underlying causes.

One of the consequences of CRH hypersecretion in depression and anorexia nervosa is hypercortisolism. Although we have shown that the mechanism of hypercortisolism in these disorders is distinct from that in Cushing's disease, the magnitude of the hypercortisolism can be as severe. In fact, because our data strongly suggest that patients with anorexia nervosa do not manifest glucocorticoid resistance, it is our sense that the consistently profound hypercortisolism in underweight anorexics would ordinarily produce the physical stigmata of Cushing's syndrome if only there were sufficient underlying biologic substrate. Hence, anorexia nervosa may constitute the only known form of centrally mediated Cushing's syndrome. In light of the fact that glucocorticoid receptors are located in many strategic locations in brain to influence a variety of behavioral and physiologic processes, it seems essential that we explore the functional consequences of hypercortisolism, especially in anorexia nervosa, but as well in depression. This is especially so because recent data strongly suggest that a principal biologic role of the glucocorticoids during physical and emotional stress is to terminate the stress responses (e.g., inhibit the CRH and LC-NE systems). In this regard, one can conceptualize depression as an illness in which there is inadequate counter-regulation by hypercortisolism of both CRH and LC-NE activation, whereas in anorexia nervosa the LC-NE system seems effectively counter-regulated while the CRH neuron is even more hyperactive than in depression. Whether there is focal glucocorticoid resistance in specific brain regions in depression or anorexia remains a question for future investigation. Parenthetically, it is our sense that the long-standing suppression of LC-NE function in anorexics even after correction of weight loss reflects the prolonged suppression of this system by the prior history of profound hypercortisolism. We have previously demonstrated that the adrenal insufficiency which persists for many months after successful transphenoidal adenectomy in Cushing's disease reflects prolonged suppression of the CRH neuron by long-standing hypercortisolism.

In addition to common defects in CRH regulation and the associated hypercortisolism, these and other neuroendocrine abnormalities suggest hyperfunction of central 5HT function in anorexia nervosa and depression. Hence, we have recently shown that 5HT is a potent stimulus to in vitro CRH secretion, while this neurotransmitter could also contribute to the hypothalamic hypogonadism and hypersecretion of growth hormone common to the two disorders.

Despite pronounced similarities between depression and anorexia nervosa, these illnesses also manifest clear differences. For example, the kind of monosymptomatic obsession which consumes the anorexic is less common in depression, while depressed mood associated with intense dysphoric hyperarousal is less present in anorexia nervosa. Although many factors could account for these differences, our initial studies will focus on norepinephrine and vasopressin.

This choice reflects, in part, the fact that abnormalities in each are in opposite directions in depression and anorexia nervosa, while each has the potential to act synergistically with the common defect in CRH regulation. Moreover, the confluence of enhanced CRH and central vasopressinergic function in anorexia could theoretically contribute to the profound obsessionalism of this disorder, while concomitant activation of the CRH and LC-NE systems in depression could contribute to the greater dysphoric hyperarousal associated with this illness.

Plans for Specific Clinical Studies in the Eating Disorders and Depression:

We are about to commence a study of the effects of prolonged administration of the potent glucocorticoid antagonist, RU 486, in patients with anorexia nervosa and depression. Such a study will have relevance to many of the theoretical issues raised above. For instance, we predict that patients with anorexia nervosa and depression will show a greater 'overshoot' in pituitary-adrenal function and in CSF CRH levels than controls, further validating hyperactivity of the CRH system in these disorders. Such a study will also allow us to determine whether pathophysiological abnormalities potentially attributable to hypercortisolism resolve during RU 486 administration (e.g., decreased sensitivity of the osmoreceptor in depression, blunted prolactin responses to m-CPP in anorexia nervosa, hypothalamic hypogonadism in each disorder). We shall further assess the impact of this intervention on indices of noradrenergic and serotonergic functional activity (e.g., CSF NE, MHPG, 5HIAA) and on a variety of behavioral and cognitive parameters which may also be influenced by sustained hypercortisolism. Finally, we shall assess lymphocyte glucocorticoid receptor mRNA before and during the trial as a more definitive means of documenting that peripheral glucocorticoid resistance is not associated with either illness.

Further efforts to evaluate CRH function in patients with anorexia nervosa and depression will include assessment of the architecture of the secretion of CRH and related peptides and neurotransmitters into the CSF via continuous drainage of lumbar CSF through an indwelling lumbar drain and evaluate the relationship between these parameters and basal circadian pulsatile pituitary-adrenal function. We also hope to evaluate the potential role of CRH in the overall symptom complexes of depression and anorexia by administering a lipophilic CRH antagonist. As a corollary, we also plan to evaluate the relative role of CRH and vasopressin by acutely administering CRH and vasopressin receptor antagonists active at the pituitary corticotroph cell.

To evaluate serotonergic function in depression and anorexia nervosa, we propose to administer IV m-CPP in the lowest maximal stimulatory dose for pituitary-adrenal activation in the evening, when the HPA is normally quiescent. In light of the potent stimulatory effect we have observed for 5HT upon in vitro CRH release, m-CPP may be both an important means of evaluating the potential role of serotonin on CRH-induced hypercortisolism and also the best available peripherally administered stimulus for evaluation of the hypothalamic CRH neuron in clinical populations. In light of a stimulatory 5HT effect on vasopressin secretion, m-CPP could also function as one of several non-osmotic stimuli to be applied at various times to further evaluate the functional integrity of vasopressin secretion in our clinical populations. An additional

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strategy to be seriously considered is a therapeutic trial of the 5HT antagonist, metergoline, a short course to assess its effects on parameters such as various indices of HP function or on glucocorticoid-mediated negative feedback.

Our clinical studies in depression and anorexia nervosa will be complemented with a full program of pre-clinical investigation utilizing our capacity to apply the techniques of molecular biology, autoradiography, immunocytochemistry, and behavioral pharmacology. For instance, we plan a series of studies to explore the source of neurohormones secreted into the CSF and to determine whether the CSF plays a physiologically relevant role for parasynaptic information processing in brain. Such studies will include a variety of lesioning experiments, the ICV administration of labelled peptides, and the electrical stimulation of specific peptide-containing regions, with subsequent evaluation of anatomic distribution and receptor function. We also plan to employ in vivo autoradiographic techniques to study the effects of maternal deprivation or inescapable shock on the regulation of parasynaptic CRH and opiate receptors. In our behavioral pharmacology laboratory, we plan to evaluate the potential synergy between CRH and AVP on CNS functions such as locomotion, feeding, and extinction of aversively conditioned learning. Moreover, we shall also attempt to develop animal models of anorexia nervosa utilizing either taste aversion on mild-moderate aversive conditioning of food intake, with subsequent evaluation of centrally administered AVP or CRH antagonists, or study of underlying biologic substrates (e.g., via in situ hybridization).

Comparative Studies of Depression and Cushing's Disease:

In further studies, we shall pursue the development of additional clinical studies to facilitate the differential diagnosis of Cushing's disease and depression. These will include the application of the alpha-2 blocker yohimbine which stimulates pituitary-adrenal function via the CNS and, hence, should fail to provoke a response in patients with Cushing's disease. We shall also apply other challenges such as alprazolam, which we have shown suppresses the hypothalamic-pituitary-adrenal axis via a central rather than a peripheral mechanism. Similarly, we have commenced a comparative study applying the glucocorticoid antagonist to both depressed and Cushing's disease patients. Previous work has shown that RU 486 preferentially augments pituitary-adrenal function during times when the CRH neuron is presumably most active. An additional focus of our comparison of depression and Cushing's disease has been our studies of CSF neurohormones and neurotransmitters. Previous studies have shown significant differences in the levels of CRH and other behaviorally active peptides, with implications for both the differential diagnosis and pathophysiology of these two disorders.

Future Directions of Tissue Culture Studies:

We have previously conducted an extensive series of studies exploring the regulation of hypothalamic CRH secretion utilizing an in vitro rat hypothalamic organ culture system. We shall continue with these studies, which will be extended to include assessment of effects of psychotropic agents and the measurement of other hypothalamic hormones such as vasopressin. A major new initiative in the area of tissue culture studies will be led by Dr. Mark Smith, who has begun to develop tissue culture systems for brain regions other than the hypothalamus and to assess the response of various tissues not only by studies of secretion but also by methodologies such as Northern blot analysis and

quantitative in situ hybridization.

Future Directions of Primate Laboratory:

We have undertaken a major initiative in collaboration with Dr. Miles Herkenham and his group to explore the source and regulation of neurohormonal secretion into primate CSF. To accomplish this goal, we have developed a procedure for placing an indwelling intraventricular line in tethered primates who may be studied without chair restraint. An additional theoretical focus will be on studies to explore whether coherent physiology governs neurohormonal secretion into the CSF and, if so, whether such secretion can be construed to have a physiologically relevant function.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01090-11 BP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Central Nervous System Functional Anatomy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Miles Herkenham, Ph.D., Chief, Unit on Functional Neuroanatomy, SCN, BPB, NIMH

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Kenner C. Rice, Chemist, LN, NIDDK

COOPERATING UNITS (if any)

Neuroscience Branch, Laboratory of Pre-Clinical Pharmacology, SEH, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Clinical Neuroendocrinology, Unit on Functional Neuroanatomy

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

5.2

PROFESSIONAL:

5.2

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A sensitive method for light microscopic localization of brain receptors by in vitro autoradiography was developed previously in this laboratory. By this method we have mapped the locations of drug and neurotransmitter receptors in the brains of rats and other vertebrates, including primates. Immunohistochemistry is used to compare the locations of transmitter-specific pathways with the locations of the relevant receptors. The non-correspondence, or "mismatch" between the locations of receptors and transmitters in the opiate and tachykinin systems, as well as others, allows hypotheses about parasynaptic circuitry in the central nervous system. Physiological activation of vasopressin dynorphin pathways in the posterior pituitary altered kappa opiate receptor binding in the neurohypophysis, a site of parasynaptic communication. Current and planned work uses similar strategies to better understand the forms of functional intercellular communication.

Objectives:

Over the last decade a major thrust of neuroscience research is the identification of receptors for drugs, neurotransmitters, and other "informational substances" in the brain. An understanding of receptor function requires knowledge of the biochemistry and pharmacology as well as the neuroanatomical localization of receptors. Receptors are identified by pharmacological criteria in collaborative studies with Dr. R. B. Rothman, Laboratory of Preclinical Pharmacology, NIMH. We use immunohistochemistry to identify the neuronal circuitry that is "plugged into" these receptors. Our finding that the organization of chemically-defined pathways is different than the organization of receptor distributions led to a major proposal for the existence of endocrine actions of neurotransmitters, apart from their roles in synaptic physiology. A major objective is to use anatomical, biochemical, and molecular techniques to show the dynamic relationships between informational substances and their receptors, focusing on the corticotropin-releasing hormone (CRH) and opiate systems. We have chosen these neuropeptides because of their well-characterized roles as central regulators which elicit a coordinated and coherent series of adaptive responses to stressful or painful stimuli.

Methods Employed:

We have successfully developed an *in vitro* autoradiographic technique for visualizing drug and neurotransmitter receptors in slide-mounted tissue sections. The details of this technique were described previously (Project number Z01 MH 01090-09 LNP). Radiolabeled tracer substances can also be mapped autoradiographically after systemic or intracerebroventricular injections in order to visualize distribution channels or target sites for biologically active drugs. Chronic drug delivery is made possible by surgical implantation of osmotic minipumps and (if necessary for delivering neuropeptides which do not cross the blood-brain barrier) ventricular cannulae. *In vivo* receptor autoradiography allows competition for binding between injected ligand and endogenous ligand, to enable us to make inferences about the status of endogenous systems during physiological manipulations. Dynamic activities in specified neurotransmitter systems will be probed by the technique of *in situ* hybridization. Quantitative immunohistochemistry will be used to compare genetic expression of transmitter with levels measured in fiber terminals. Thus we are equipped to simultaneously monitor numerous aspects of transmitter function during specified physiological conditions. Facilities for precise behavioral control and measurement have been set up to permit study of animal models of stress and mental disorders.

Major Findings:

Several anatomical tools have been used to reach our stated objectives. Receptor autoradiography informs us about the relationship of neurotransmitters and their receptors only if it is done properly, that is to say, with preservation of anatomical

detail, with well-defined and validated binding conditions, and with proper selection of structures and species for demonstration of the relationships between transmitters and receptors. Likewise, transmitter distributions are accurately localized only with proper immunohistochemical techniques. We emphasize the quality of our work because that is a major reason why our work has helped to create a shift of awareness within the neuroscience community towards an appreciation of the parasynaptic mode of intercellular communication. Our work in the opiate and tachykinin systems and our use of comparative anatomy has provided the bulk of the supportive evidence for parasynaptic communication as a plausible explanation for the observation of mismatches between the locations of transmitters and receptors in brain. In addition, however, we have argued for the generality of this phenomenon by thorough analysis of a large variety of data taken from the literature, resulting in major position papers published as a book chapter and, more recently, as a 38-page Commentary in *Neuroscience*.

We have begun an aggressive program of functional approaches to an understanding of parasynaptic function in brain by combining behavioral pharmacological techniques with neuroanatomy. After using subtype-selective binding conditions to show that opiate receptors in the pituitary are exclusively kappa, we manipulated levels of endogenous opioid peptides by chronically dehydrating or salt-loading rats, resulting in massive co-release of vasopressin and dynorphin in the neural lobe. This manipulation resulted in a down-regulation of neural lobe opiate receptors, as measured by binding kinetics performed on slide-mounted tissue sections. There are no synapses in the neural lobe, and these receptors appear to be located on nerve terminals of neurosecretory axons as well as on pituitocytes (modified astroglia), thus qualifying as parasynaptic receptors.

In a different approach, we are focusing on CRH and its role in the production of a coordinated series of centrally mediated events collectively termed the stress response. Projects are underway to examine this response in rats, marmosets, squirrel monkeys and rhesus monkeys. Each species offers potentially important insights into the stress response and concomitant depression in humans.

In coordination with our objectives to obtain a greater understanding of the neuroanatomical mechanisms of stress response, our recently-created behavioral pharmacology laboratory has developed methods to precisely and rapidly establish dose-effect functions comparing the effects of stress-related peptides on a variety of different types of behaviors. To date the effects of CRH, arginine vasopressin, oxytocin, and angiotensin II have been established with the goal of assessing potential interactions between these peptides. In addition, dose-response functions for ether-induced stress have been established in order to facilitate correlations between potential endogenous mechanisms of stress and behavioral effect. Lastly, mathematical modelling procedures have been refined to allow the determination of the effects of very low doses of agents.

Significance to Biomedical Research and to the Program of the Institute:

The visualization by autoradiographic techniques of opiate receptor locations throughout the CNS has greatly advanced our appreciation of the richness of opiate functions in normal physiology and has led to new insights into receptor-mediated brain processes. Receptors that are not located at sites of synaptically released transmitter may be mediating transmitter action of a more hormonal nature. This parasynaptic or endocrine organization of the brain has many implications for biological psychiatry. For example, centrally acting drugs exert pervasive effects after diffusion through extracellular spaces and, thus, may mimic the mode of action of endogenous neurochemicals far more than was previously thought. Further work examining parasynaptic mechanisms should help to elucidate pathophysiological mechanisms in psychiatric illness and provide a foundation for better understanding of information transfer and consolidation in the brain. When these phenomena are understood in better detail, significant improvements can be expected in the approach to treatment of neuropsychiatric disease.

Proposed Course of the Project:

With our growing appreciation that many receptors are nonsynaptically located and, therefore, may mediate parasynaptic intercellular communication in an endocrine fashion, we will explore several lines of pertinent inquiry. We propose to trace the movement of inert as well as biologically active peptides through the cerebrospinal and extracellular fluids. Other neuroanatomical work will use comparative and developmental approaches in several neurotransmitter/receptor systems (we will focus on the opiate and CRH systems) to gain insights into the rules of organization of the respective distributions. Correlative studies of the distributions of relevant molecules, such as synthesizing and degradative enzymes, and neural pathways identified by histochemical and physiological means will serve to generate hypotheses about functional operations within the systems. Quantitative autoradiographic techniques will be used to show dynamic relationships between transmitters and parasynaptic receptors. Molecular probes and *in situ* hybridization will be used to show the locations and physiological regulation of cells expressing the relevant markers. Similar studies carried out in human tissues will allow inferences to be made about the pathophysiology of psychiatric illnesses.

We are also investigating the functional roles of selected neuropeptides as components of central neuroendocrine systems. We plan studies in which transmitter synthesis, transport and release and receptor regulation are concurrently studied under controlled behavioral or physiological manipulations. As a result of such studies, it may be possible to develop animal models of psychiatric disorders, such as depression, with known endocrine abnormalities detected by aberrant fluctuations of peptide hormones in the cerebrospinal fluid or individual brain loci. Our choice of primates for many of these studies is based on the availability of

large volumes of CSF for sampling studies, on the ability to take advantage of complex social behaviors (such as pair bonding in marmosets and distress vocalizations in squirrel monkeys), and the applicability of the results to similar human conditions.

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mosaic: II. Patch and matrix directed mesostriatal dopaminergic and non-dopaminergic systems. J. Neuroscience, in press.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00180-05 BP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychobiology and Treatment of Menstrually-Related Mood Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David R. Rubinow, M.D., Chief, Unit on Peptide Studies, BPB, NIMH

P. Schmidt, BPB, NIMH; M.C. Hoban, BPB, NIMH; K. Denicoff, BPB, NIMH; G. Merriam, ERRB, NICHD; R. Elin, CPD, CC, NIH; H. Weingartner, BPB, NIMH; B. Both-Ortmann, BPB, NIMH; F. Putnam, St. Elizabeth's Hospital; D. Raben, ERRB, NICHD; N. Hall, George Washington University; E. Bou, CC, NIH; N. Rosenthal, CPB, NIMH; R. Anderson,

COOPERATING UNITS (if any)

BPB, CPB, NIMH; ERRB, NICHD; HEB, NHLBI; CPD, CC, NIH; St. Elizabeth's Hospital; George Washington University

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Peptide Studies

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The occurrence of dramatic changes in mood, behavior, cognition and somatic functioning in some women in relation to the menstrual cycle has recently been the focus of a great deal of public scrutiny. This project is designed to study the psychobiology and treatment response of women with well-defined menstrually-related mood disorders. The longitudinal screening methods developed by this group are capable of distinguishing women with menstrually-related mood syndromes from those who only believe that they have such a syndrome. With these methods, we have identified the following: 1) menstrual cycle phase dependent changes in perception and cognitive performance in patients with menstrually-related mood disorders but not controls; 2) an increased tendency to dissociate in patients versus controls; 3) an increased prevalence of abnormal basal and stimulated thyroid measures in patients; 4) a higher than expected frequency of hypoglycemic episodes following glucose tolerance testing in women with premenstrual syndrome irrespective of menstrual cycle phase; and 5) preliminary evidence of the efficacy of alprazolam in the treatment of premenstrual syndrome relative to placebo or other treatments employed. The goals of this project are to detect and accurately describe menstrually-related mood disorders, explore their pathophysiology and response to pharmacological and environmental manipulation, and to document the relationship between reproductive endocrine change and disorders of mood as a way of further investigating the neurobiology of psychiatric illness.

I. Project Description

A. Objectives

This project has as its main intent the selection of subjects with carefully documented menstrually-related mood changes who can then undergo psychological and biological evaluation as well as participate in double-blind, placebo-controlled trials of several widely prescribed treatment modalities.

B. Methods Employed

1. Subjects

a. Subjects are self- and physician-referred women between the ages of 18 and 55 who meet study criteria as described in detail in Project #Z01 MH 00180-03 BP.

b. Normal controls for this study include women with no complaints nor evidence of menstrually-related mood disorder and who are without primary psychiatric illness, and women who have complaints of, but no visual analogue scale evidence of, menstrually-related mood changes.

2. Procedures

Phase 1. An extensive screening phase that has been described in detail in Project Z01 MH 00180-02 BP.

Phase 2. This is an intensive psychobiological evaluation phase for patients meeting entry criteria for the study.

a. Patients are given a thorough physical and laboratory examination in order to rule out the presence of unknown medical illness.

b. Ongoing studies of longitudinally obtained basal and stimulated hormonal levels have been previously described in Project #Z01 MH 00180-03 BP. In addition, we are performing the following studies:

1) Psychometrics: Previously described cognitive and mood self-rating batteries are currently being supplemented by evaluation of the frequency of and response to life events over the course of the menstrual cycle (M.C. Hoban, Dr. P. Schmidt), comparison of performance on the Raven Progressive Matrices Tests during the symptomatic and symptom-free phases of the menstrual cycle, determination of menstrual cycle phase-dependent associations, and longitudinal self-evaluation of mood state satisfaction (Drs. H. Weingartner and F. Putnam).

2) Diet: In collaboration with Dr. N. Rosenthal and E. Bou, we are evaluating dietary patterns over the course of the menstrual cycle in order to test hypotheses about altered carbohydrate or salt intake in menstrually-related mood disorders.

3) Glucose tolerance test: Because of reports of altered carbohydrate tolerance in premenstrual syndrome, we are performing glucose tolerance tests in patients with menstrually-related mood disorders in both cycle phases (Dr. K. Denicoff).

4) Corticotropin releasing hormone (CRH) stimulation tests: With Dr. D. Raben, we are performing CRH stimulation tests during the follicular and luteal phase in patients and controls to see if there is a biological marker for the reported altered stress sensitivity in women with premenstrual syndrome.

5) Clonidine stimulation tests: Because of the hypothesized role of endogenous opiate withdrawal in the precipitation of menstrually-related mood symptoms and a preliminary report of the efficacy of clonidine in the treatment of premenstrual syndrome, we have performed clonidine infusions during the symptomatic and asymptomatic states in order to assess menstrual state-related symptomatic and endocrine response.

6) Immune system: In light of reports of both altered T-cell function as a function of estrogen levels and abnormal allergic responses in women with premenstrual syndrome, we are investigating T-cell function in relation to menstrual cycle phase in women with premenstrual syndrome and normal controls, in collaboration with Dr. N. Hall.

Phase 3. This is a multi-modality treatment phase for patients who have completed Phase 2. Double-blind, placebo-controlled crossover evaluation of progesterone, medroxyprogesterone acetate, pyridoxine, carbamazepine, alprazolam, and nalmefene are currently being conducted. Rationales for the selection of these particular compounds have been previously described. Additionally, protocols have been submitted for the evaluation of the anti-progesterone agent RU 486 and "square wave" progesterone withdrawal in the treatment of premenstrual syndrome. It is believed that these protocols will provide critical information regarding the role of the reproductive endocrine profile during the luteal phase in the production of mood changes that occur during the latter phase of the menstrual cycle.

C. Findings

A variety of menstrual cycle phase-dependent psychological and cognitive changes have been identified in women with premenstrual syndrome compared with controls. Thus, women with premenstrual syndrome tend to report a greater number of negative life events and a smaller number of positive life events during the luteal phase, and further, experience the same events as more negative during the luteal phase. Baseline measures of the tendency to dissociate are higher in women with premenstrual syndrome than controls, and are higher in the luteal than in the follicular phase. Cognitive performance, as measured by the Raven Progressive Matrices Text, is more impaired in premenstrual syndrome women during the luteal phase while control women tend to show more impaired performance during the first test administration, irrespective of menstrual cycle phase. State complacency scale measures of affective change reveal that change along dimensions of mood state stability, sense of control, and satisfaction with mood state can occur independently in

women undergoing mood state switches. These data, along with data from our previously described endocrine studies, are consistent with the hypothesis that premenstrual syndrome represents a biologically facilitated state change, with the altered cognitive and perceptual characteristics a product of the altered state, rather than the normal reproductive endocrine changes.

Efforts to characterize the biology of premenstrual syndrome have included the performance of TRH and CRH infusions, as well as performance of glucose tolerance tests and measurement of progesterone metabolites. We have extended our earlier demonstration of a high prevalence of abnormal TSH responses to TRH in patients with premenstrual syndrome, but have also demonstrated that, despite reports to the contrary, premenstrual syndrome is not simply hypothyroidism and the occurrence of thyroid autoantibodies in women with premenstrual syndrome is a rare rather than a common event. Glucose tolerance testing revealed a high frequency of hypoglycemic episodes in women with premenstrual syndrome irrespective of menstrual cycle phase. However, the symptoms experienced were typical of hypoglycemic attacks and were atypical of the symptoms ordinarily experienced during the luteal phase. CRH tests have been performed in both menstrual cycle phases in eight patients and eight controls to date. The results of this testing are not currently available. In collaboration with Dr. R. Anderson, several progesterone metabolites have been identified in the plasma of women with premenstrual syndrome, but not in controls. We are currently pursuing this finding with larger groups of patients and controls.

Studies of alterations in immune function and osmoregulatory hormones over the course of the menstrual cycle in patients and controls have been completed, although the final results have not been obtained at present. Initial experience with the 11 patients participating in a double-blind, placebo-controlled trial of alprazolam are encouraging and superior to those observed in similar studies of vitamin B6, progesterone, and the opiate antagonist nalmefene. Finally, we observed that luteal phase-related increases in appetite are observed in both patients and controls, although the increase in appetite in patients is threefold greater than that observed in controls. Additionally, the changes in appetite observed in patients are highly correlated with changes in mood, while the same is not observed in the controls.

D. Proposed Course of Project

With a group of well-defined patients, we hope to explore the natural course of menstrually-related mood disorders as well as their phenomenology and biological correlates in relation to treatment response. Early endocrine findings will be pursued and specific hypotheses regarding the etiology of premenstrual syndrome (e.g., endorphin addition/withdrawal, carbohydrate intolerance, electrolyte dysregulation) will be tested with endocrine challenge studies. Treatment protocols will be completed, and evaluation of putative therapeutic agents will be undertaken. Cognitive testing will be continued with the addition of distractors during testing. We will explore the state- and stress-related characteristics of premenstrual syndrome through implementation of state-dependent learning paradigms and continued performance of CRH stimulation testings. Protocols for the administration of RU 486 and

"square wave" progesterone withdrawal will help define, better than any currently available evidence, the nature of the relationship between luteal phase endocrine events and mood changes. These protocols have great explanatory potential because they effectively blind the patient to her position in the menstrual cycle, a methodological problem that was previously uncorrectable. We wish to expand our investigation of the effects of menstrual phase on mood to include patients hospitalized at the Clinical Center with major affective disorder, panic anxiety disorder, and anorexia-bulimia, as well as patients with hereditary angioedema. Our early experience with a number of women with these disorders suggests that symptoms may be exacerbated or may cluster during the premenstruum; these clinical impressions require prospective confirmation. Finally, studies of women with post-partum and menopausal depression are being designed.

E. Significance to Biomedical Research and the Program of the Institute

Despite the current lack of clear understanding of the nature of the relationship between mood disorders and the menstrual cycle, numerous studies of this phenomenon suggest its importance to the psychiatrist on many levels: practically (as a problem about which the psychiatrist may be called to consult or as a factor which may influence the course of the treatment of patients); heuristically (as a model for learning about state changes, a process of clear relevance to studies of other mood state disorders such as manic-depressive illness or panic anxiety disorder); and conceptually (as a potential means for providing biological-phenomenological isomorphs and further understanding the role of entrainment in episodic or cyclic psychiatric disorders). Menstrually-related mood disorders in their own right are important to better understand, if only for the fact that there are large numbers of women who feel that they suffer from such syndromes and seek treatments that are unproved and potentially dangerous. In addition, it would appear that menstrual cycle phase is a variable that has been all too frequently ignored in studies of traditional psychiatric and medical illnesses. It is our belief, therefore, that this project will provide information that will be of immediate clinical relevance and that will further our understanding of the complex relationship between endocrine system activity and mood.

PUBLICATIONS

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- Rubinow, D.R., Hoban, M.C., and Grover, G.N.: Menstrually-related mood disorders. In Nerozzi, D., Goodwin, F.K., and Fragese, G. (Eds.): Hypothalamic Dysfunction in Neuropsychiatric Disorders. New York, Raven Press, 1987, pp. 335-346.
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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00181-04 BP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hormonal Studies of Affective Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David R. Rubinow, M.D., Chief, Unit on Peptide Studies, BPB, NIMH

P. Sunderland, M.D., LCS, NIMH; M. Linnoila, M.D., LCS, NIAAA; M.; S. Bracha, M.D., M, SMRC; S.R.B. Weiss, Ph.D., BPB, NIMH; W. Kaye, M.D., Univ. of Pittsburgh; J. Crawley, Ph.D., NSB, NIMH; K. Denicoff, M.D., BPB, NIMH; P. Hauser, M.D., BPB, NIMH; J. Hill, Ph.D., NSB, NIMH

COOPERATING UNITS (if any)

BPB, LCS, NSB, NIMH; LCS, NIAAA; NCI; M, SMRC; SUNY, New York; University of Pittsburgh

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Peptide Studies

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of somatostatin and cortisol in relation to affective and other neuropsychiatric disorders have continued. Additionally, further studies of the mechanisms of interleukin-2-induced neurotoxicity have been performed in rodents.

A) Somatostatin - CSF somatostatin has been measured in an expanded group of neuro-psychiatric patients including patients with multiple sclerosis and neurological controls, patients with alcoholism, pathological gambling, and Huntington's dementia. Significant correlations were observed between somatostatin and performance on the Wechsler Memory Scale, supporting our earlier observations (and perhaps the clinical relevance) of decreased somatostatin levels in patients with neuropsychiatric disorders characterized by cognitive impairment. Studies of the regional concentrations of brain somatostatin in post-mortem schizophrenics, suicide victims, and accident victims have been completed with the sample code not yet broken.

B) Cortisol - A salivary cortisol method, developed and validated in our laboratory, has been used to longitudinally follow patients with affective and anxiety disorders. This method shows great promise for studies of ambulatory patients with affective disorder.

B) Interleukin-2 - No changes in blood-brain-barrier permeability were observed in rats treated with interleukin-2, irrespective of the size of the tracer employed. These findings contradict previous reports and leave unresolved the mechanism of lymphokine-induced neurotoxicity.

I. Project Description

A. Objectives

The goal of this project is to study neuroendocrine and immune soluble products in patients with neuropsychiatric disorders in order to expand our understanding of the mechanisms and significance of reported abnormalities in somatostatin, cortisol, and immune system activity in affective illness.

B. Methods Employed

1. Subjects

a. Subjects include inpatients on NIMH clinical units meeting criteria for major depressive disorder, Alzheimer's disease, Huntington's dementia, pathological gambling, anorexia nervosa, and bulimia as well as patients with substance abuse disorder (alcoholism), Cushing's syndrome, and multiple sclerosis.

b. Normal controls are volunteers selected from the normal volunteer program at the NIH. Neurological controls were employed for studies of patients with multiple sclerosis.

2. Procedures

Lumbar punctures are performed to obtain CSF samples for somatostatin, CRF, and other related CNS peptides/neurotransmitters. Regional analysis of brain somatostatin is performed in post-mortem human brain samples. Brain slices in animals are punched and assayed for somatostatin content. Whole brains are dissected and radioactivity measured in animal lymphokine studies.

II. Findings

A. Somatostatin

Preliminary evidence obtained in collaboration with Dr. P. Hauser suggests both a decrease in CSF somatostatin in patients with multiple sclerosis relative to neurologic controls, as well as an increase in CSF somatostatin in patients with multiple sclerosis during clinical remission. In collaboration with Dr. M. Linnoila, we observed no syndromal alterations in CSF somatostatin in patients with alcoholism, pathological gambling, or Huntington's dementia. However, we did observe a highly significant correlation between CSF somatostatin and performance on the Weschler Memory Scale in patients with Huntington's dementia, a finding previously observed by us in Alzheimer's patients, and one that further suggests the relevance of diminished somatostatin levels in patients with neuropsychiatric disorders characterized by cognitive impairment. In collaboration with Dr. W. Kaye, we have observed normal CSF somatostatin concentrations in patients with anorexia nervosa (at low weight and after weight recovery) and in bulimic women. However, when normal weight bulimics stop bingeing, they display a modest but significant increase in CSF somatostatin. CSF somatostatin was not related to plasma growth hormone levels, but did show relationships to the

hypothalamic-pituitary-adrenal (HPA) axis. Thus, as we previously demonstrated, a significant positive relationship was observed in healthy controls between CSF somatostatin and CSF CRH. Additionally, in underweight anorectics, CSF somatostatin was negatively related to both 24-hour urinary free cortisol and plasma cortisol levels after dexamethasone. The differences in the relationship between somatostatin and the HPA axis in anorectics and bulimics may constitute or reflect pathophysiological distinctions between these disorders.

B. Cortisol

We have demonstrated the practical utility of the salivary cortisol measure in the evaluation of the function of the hypothalamic-pituitary-adrenal axis. Appropriate increases and decreases in salivary cortisol were observed during CRH stimulation tests and dexamethasone suppression tests, respectively, and circadian alterations were also observed in both normal volunteers and patients with affective illness. Longitudinal studies of several patients with affective illness prior to and during treatment with carbamazepine revealed no evidence of carbamazepine-induced cortisol hypersecretion as indicated by elevated salivary cortisol (and hence free cortisol) levels.

C. Interleukin-2

In collaboration with Drs. K. Denicoff and J. Crawley, we observed no alterations in blood-brain-barrier permeability in animals treated with interleukin-2 (IL-2) demonstrating behavioral toxicity. Both high and low molecular weight radio tracers (albumin and alpha-amino isobutyric acid) were employed, but no increased brain accumulation was seen with either tracer at any time-point during the week of IL-2 administration. These findings contradict those previously reported in mice. Finally, in collaboration with J. Hill, we were unable to demonstrate the induction of brain IL-2 receptors following IL-2 treatment in rodents. Thus, the mechanism of the IL-2-induced neuropsychiatric toxicity that we observed in humans remains unresolved.

III. Proposed Course of Project

We hope to: 1) examine the effects of electrical kindling and learned helplessness on brain somatostatin and m-RNA in rodents; 2) develop assay techniques to permit evaluation of the contribution of somatostatin fragments to the total syndromal alterations in CSF somatostatin observed; 3) employ salivary cortisol measures to characterize the mood state switches that occur in patients with affective illness in ambulatory settings; 4) explore alternative mechanisms for enabling somatostatin to pass the blood-brain-barrier so as to be able to test its efficacy as a treatment for cognitive disturbances in patients with Alzheimer's disease and affective illness; and 5) continue investigations of the neuroimmunoendocrine concomitants and inducers of impaired cognitive performance.

IV. Significance to Biomedical Research and the Program of the Institute

Depression-related dysregulation of somatostatin and cortisol may provide a window into the central neurochemical lesions responsible for depression.

Further, specific behavioral or physiological disturbances (e.g., cognitive impairment or cortisol dysregulation) may be products of abnormal neuroendocrine activity. It may prove to be the case that depression-related reductions in somatostatin are mechanistically relevant to depression-related disturbances in hypothalamic-pituitary-adrenal activity, the most commonly reported biological abnormality in depression. Determination of the mechanisms of the profound behavioral and cognition altering effects of interleukin-2 would fill a major gap in our knowledge of the ways in which the immune system can regulate central nervous system activity. Further study may not only enhance our knowledge of the neurobiology of depression, but may, as well, more generally inform us about the relationship between hormones and human behavior.

PUBLICATIONS

Rubinow, D.R., Post, R.M., Davis, C.L. and Doran, A.R.: Somatostatin and GHRH: Mood and behavioral regulation. In Nerozzi, D., Goodwin, F.K. and Fragese, G. (Eds.): Hypothalamic Dysfunction in Neuropsychiatric Disorders. New York, Raven Press, 1987, pp. 137-152.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00182-04 BP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Medicine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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Dr. David Pickar, Clinical Neuroscience Branch, NIMH

Dr. Steven Rosenberg, Surgery Branch, NCI

Dr. Pim Brouwers, Georgetown University, Washington, D.C.

Dr. Clifford Lane, Laboratory of Immunoregulation, NIAID

COOPERATING UNITS (if any)

BPB, NSB, LPP, CPB, NIMH; SB, PB, FCRF, NCI; LIR, LCI, NIAID; CCM, CC; OD, CE, A&R, NIADDDK; MD, CB, NHLBI; St. Michael's Hosp., Toronto; Georgetown Univ.

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Peptide Studies

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Eight protocols are currently active and conducted out of the Consultation-Liaison Service-based behavioral medicine research program. These protocols examine the phenomenology and biological correlates of illness or treatment-induced mood, behavioral, and cognitive changes. The protocols address such areas as: a) the effects of previous psychiatric history on the psychiatric morbidity associated with certain diseases and their treatment; b) the psychiatric phenomenology of certain diseases and their treatment; c) the treatment response characteristics of psychiatric disorders associated with diseases or their treatment; d) biochemical factors that may serve as predictive diagnostic markers for illness or for treatment-associated mood/behavioral or cognitive syndromes; e) the effects of mood state alterations on immunologic function. Significant findings to date include demonstration of the following: 1) significant neuropsychological cognitive impairment in patients with AIDS compared with seropositive patients, chronically medically ill patients, or controls; 2) significant cognitive and affective deterioration following removal of thyroid hormone replacement, with no differential effects of T_4 and T_3 on mood and cognition; 3) absence of evidence of acute immune changes following hypnotically-induced affective states; 4) a significant effect of dose on interleukin-2-induced neuropsychiatric toxicity; 5) preliminary confirmation of alpha-delta intrusion in patients with fibromyalgia; 6) preliminary evidence of exaggerated response to novelty in patients with chest pain and normal coronary arteries.

Other Professional Personnel (continued)

Dr. Jacob Robins, Clinical Endocrinology Branch, NIADDDK
Dr. Stanley Pillemer, Arthritis & Rheumatism Branch, NIADDDK
Dr. Richard Cannon, Cardiology Branch, NHLBI
Dr. Russell Joffe, St. Michael's Hospital, Toronto, Canada
Dr. Nicholas Hall, Georgetown University, Washington, D.C.
Dr. Peter Schmidt, Biological Psychiatry Branch, NIMH
Dr. Daniel Longo, Frederick Cancer Research Facility, NCI

I. Project Description

A. Objectives

This project has as its main intent the identification of mood and cognitive symptoms that appear in the context of specific medical illnesses and their treatment, determination of the relationship between these symptoms and both the primary medical disorder and prior psychiatric history, and utilization of the occurrence of these symptoms in a medical context as models for the occurrence of similar symptoms in a primarily psychiatric context.

Protocols

Active:

- 1) Neuropsychiatric dysfunction in patients with Acquired Immune Deficiency Syndrome (AIDS).
- 2) The effect of thyroid replacement and withdrawal on cognition and mood in patients with carcinoma of the thyroid.
- 3) Assessment of neuropsychiatric concomitants of metoclopramide administration.
- 4) The effect of hypnotically-induced affect on immune function in normal subjects.
- 5) Neuropsychiatric effects of alternate day steroid administration in patients with systemic lupus erythematosus.
- 6) The cognitive and behavioral effects of interleukin-2/lymphokine activated killer cell therapy.
- 7) Mood and cognitive toxicity of gamma-Interferon administration in seropositive patients.
- 8) Fibromyalgia.

Completed:

- 1) A prospective study of the behavioral, cognitive and neurochemical effects of chronic, systemically-administered corticosteroids.
- 2) The correlation of hypnotizability, dissociation, and absorption in normal subjects.

Planned:

- 1) Conditioned immunosuppression and immunoenhancement in cancer patients.
- 2) Development of endocrine correlates of the "intensive care unit syndrome".

B. Methods Employed

1. Subjects

a. Subjects are NIH patients who are referred for participation in these protocols by collaborators from the Institute responsible for the primary care and treatment of these patients.

b. Controls for the individual studies are selected in a way that allows for stratification of populations with respect to the relevant variables under study.

2. Procedures

a. Psychiatric Diagnostic Evaluation

The primary methodology employed is that of evaluating the psychiatric history of all subjects and their families utilizing a semistructured psychiatric interview, the Schedule for Affective Disorders and Schizophrenia (SADS-L), which provides information from which an RDC diagnosis can be made.

b. Longitudinal Evaluation

Most studies utilize a "self as own control" design employing longitudinal assessment of mood ratings, physical symptoms, and cognitive performance. Detailed description of methodologies employed can be found in Project #Z01 MH 00182-02 BP.

c. Laboratory Assessment

Urine and/or blood samples are collected in order to permit evaluation of those biological substances believed to be related to the development of affective or cognitive disturbances.

3. Findings

1) We have replicated earlier findings of cognitive impairment in AIDS patients without evidence of central nervous system opportunistic infection. Similar impairment was not observed in seropositive patients, controls, or patients with chronic active hepatitis (a chronic medically ill control group) (collaborator: Dr. C. Lane and P. Brouwers).

2) In addition to providing the first description of neuropsychiatric toxicity of interleukin-2 (IL-2) (during systemic administration), we have observed the following: increased incidence of toxic effects with high dose IL-2; absence of a relationship between prior personal or family psychiatric history and the likelihood of developing IL-2-induced neuropsychiatric changes; a clear latency between initiation of treatment and development of neuropsychiatric side effects, suggesting that a secondary rather than a direct effect of IL-2 is being observed; significant decreases in ACTH but not cortisol in association with IL-2 administration (collaborators: Drs. K. Denicoff and S. Rosenberg).

3) Preliminary analysis of a comprehensive immune profile performed on blood samples drawn prior to, during, and subsequent to a hypnotically-induced affective state change in highly hypnotizable patients under four different conditions has revealed no obvious changes in immune variables, including natural killer cell activity, response to mitogen stimulation, and IL-2 concentrations. These data will help to interpret immune alterations reported to occur during major depressive episodes (collaborators: Drs. N. Hall and P. Schmidt).

4) No differential effects of T_3 and T_4 were observed on mood or cognition in thyroidectomized patients post carcinoma of the thyroid. However, significant differences were observed in mood and cognition between either treatment and the absence of thyroid hormone replacement; specifically, marked increases in anxiety and depression, along with decreased cognitive functioning, were observed soon after removal of thyroid hormone. These data provide the first prospective, longitudinal demonstration of the effects of thyroid hormone on mood and cognition in nonpsychiatric patients. Additionally, a high prevalence of past history of affective disorder was observed in the group who were most severely symptomatic during thyroid hormone withdrawal (collaborators: Drs. J. Robins and K. Denicoff).

5) Lactate infusions performed in a small group of normal volunteers to date suggest that the panic attacks that we observed during lactate infusion in patients with chest pain and normal coronary arteries may represent an unusual response to novelty rather than a special vulnerability to lactate. We are continuing these studies in order to further define the phenomenological and biochemical similarity between patients with chest pain and normal coronary arteries and patients with major anxiety disorders (collaborators: Dr. R. Joffe, P. Schmidt, and R. Cannon).

6) Early work with patients with fibromyalgia has confirmed the presence of the alpha wave intrusion during sleep. Attempts to demonstrate these findings in a larger group of patients with fibromyalgia and compare them with arthritic controls and normal volunteers will be undertaken in order to determine whether the symptoms and EEG abnormality in fibromyalgia are specific to that syndrome or merely secondary to disturbed sleep (collaborators: Dr. S. Pillemer and K. Denicoff).

D. Proposed Course of Project

The studies noted above will be continued until adequate numbers of subjects are obtained. Attempts to identify neuroendocrine correlates of or biologic mechanisms for the lymphokine-induced neuropsychiatric disturbances have been initiated. Studies investigating the immunoregulatory potential of hypnotically-induced mood states and of the phenomenologic, somnographic, and treatment response characteristics of patients with fibromyalgia have similarly been initiated. Completion of these descriptive studies should permit design of focused investigations of the neurobiology of specific mood, behavioral, and cognitive disorders. Finally, studies are currently being undertaken to investigate the psychobiology of pathological grief in spouses of cancer patients, describe the prevalence of major anxiety disorders in patients with Graves' disease, describe the development and endocrine concomitance of the "intensive care unit syndrome", and condition immunosuppression and immunoenhancement in cancer patients.

E. Significance to Biomedical Research and the Program of the Institute

The studies in this project are hypothesis-generating as well as hypothesis-testing. Thus, they should not only help to expand the behavioral phenomenology of many medical disorders, but should, as well, suggest optimal studies for the application of modern neuroscientific techniques to disorders of regulation of

mood and cognition. Detection of conditioned immunosuppression in patients should have profound effects on both our understanding and treatment of many medical disorders. The utilization of medical disorders as models for the development of mood and cognitive disturbances in the context of biological dysregulation should clarify the meaning of these biological alterations already observed in psychiatric disorders.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00147-12 BP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiological Effects of Brain Peptides and Other Behavioral and Psychoactive Compounds

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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 Lorna Estal, Visiting Fellow, BPB, NIMH; R.J. Weber, Sr. Staff Fellow, SNB,
 NINCDS; K.C. Rice, LC, NIADDK; B. DeCosta, Visiting Fellow, LC, NIDDKD;
 A.A. Hagen, American U.; T. Seeger, Pfizer Central Research, Groton, Ct.; H.D.
 Everist, Guest Worker, BPB, NIMH; C.C. Chiueh, Staff Fellow, LCM, NIMH;
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 D. Nutt, LCS, NIAAA

COOPERATING UNITS (if any)

BPB, CNB, LCM, NIMH; LC, NIADDK; LC, NIDDKD; LCS, NIAAA; SNB, NINCDS; Pfizer Central Research, Groton, Ct., American University, Washington, D.C.

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Biological Psychiatry Branch

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2.5

PROFESSIONAL:

1.5

OTHER:

1

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Phencyclidine produces behavioral excitation through a dopamine independent mechanism in the nucleus accumbens, probably by blocking the excitatory amino acid NMDA receptor. Motor asymmetries produced by phencyclidine are caused by activation of pathways projecting caudally from the substantia nigra. Opiate-induced suppression of immune function appears to be mediated, at least in part, through the periaqueductal gray matter. The periaqueductal gray matter in general seems to be an important region of the brain for regulating the immune function. Nicotine produces behavioral excitation by activating nigrostriatal and mesolimbic dopamine pathways. Locomotor activation produced by amphetamine and cocaine in the nucleus accumbens is mediated through activation of a variety of efferent pathways projecting to the striatal fundus, ventral pallidum, zona reticulata, and the medial and lateral olivary complex. Kappa opiate receptors modulate locomotor behaviors as well as a variety of homeostatic mechanisms. The depressant actions of kappa opiate agonists are not produced through the opiate receptor but appear to be nonspecific.

I. Project Description

A. Objectives

1. Nicotinic Modulation of Nigrostriatal and Mesolimbic Dopamine Functions

We have previously reported the presence of stereospecific, saturable, and reversible binding of [³H]nicotine to rat brain sections, which is of high affinity and is selectively displaced by nicotinic agonists including acetylcholine. Autoradiographic analyses revealed heavy nicotine labeling in a variety of thalamic nuclei as well as the cortex and substantia nigra. A substantial concentration of nicotine receptors was also found in the nucleus accumbens and striatum. Subsequent lesion studies revealed that nicotine receptors are localized on both the perikarya as well as terminals of the mesolimbic and nigrostriatal dopamine neurons. The purpose of these studies was to determine if any of the behavioral effects of nicotine could be mediated through either of these dopamine pathways.

The rotational model was utilized in order to assess the effects of nicotine on nigrostriatal dopaminergic function. Rats were lesioned unilaterally in the medial forebrain bundle with 6-hydroxydopamine. Animals prepared in this manner rotate ipsilaterally to the lesion following indirectly acting sympathomimetics like amphetamine and contralaterally to directly acting sympathomimetics like apomorphine. Following recovery, the animals were injected subcutaneously with 0.5, 0.25 or 0.125 l-nicotine tartrate and placed in automated rotometers. In subsequent studies, interactive effects between nicotine and amphetamine and the ability of mecamylamine to antagonize the effects of nicotine were also studied. Since nicotine has initial behavioral depressant actions to which animals become tolerant, it was of interest to examine the effects of chronically administered nicotine on rotational output in lesioned rats.

Since the mesolimbic dopamine system appears to be involved in modulating locomotor activity, it was conceivable that the locomotor excitatory effects of nicotine were mediated through this system. Rats were implanted with cannulae guides aimed for the nucleus accumbens and the ventral tegmental area which are the brain regions containing the terminals and perikarya of the mesolimbic dopamine pathway, respectively. Following recovery, the animals were injected in these brain regions with cytisine, a potent nicotinic agonist, and observed for alterations in locomotor activity. Various pharmacological controls were utilized to ascertain whether the effects of these manipulations were specific to the dopaminergic and nicotinic systems.

We have previously found that the dopaminergic neurons of the substantia nigra zona compacta are excited by nicotine and acetylcholine. The dopaminergic perikarya in the zona compacta also possess high-affinity nicotine binding sites and intense acetylcholinesterase activity which are consistent with a cholinceptive role. In previous studies we utilized retrograde tracing with histochemical visualization of cholinergic neurons to demonstrate that pedunculopontine efferents project to the substantia nigra zona compacta. In the present studies, kainic acid was micro-infused into the tegmental region in order to stimulate local cholinergic perikarya. A functional activation of cholinergic input into the substantia nigra was assessed with extracellular recording of dopaminergic neurons in the zona compacta.

2. Functional Outflow of the Nucleus Accumbens

It is certain that the nucleus accumbens represents a critical focus for the excitatory effects of sympathomimetic compounds. Injections of indirectly- as well as directly-acting dopaminergic agonists into this structure increase locomotor output, while 6-OHDA lesions attenuate the excitatory effects of amphetamine and cocaine. Recently, Swerdlow et al (Brain Res., 1986; 306:141-143) have demonstrated that the efferent pathway from the nucleus accumbens to the subpallidal region appears to serve as one important output of mesolimbic activity for the expression of locomotor behavior. Whether the pallidal output is solely involved in translating the sympathomimetic activation of the nucleus accumbens into locomotor excitation remains to be determined. The purpose of this investigation was to assess the functional outputs of the nucleus accumbens following direct activation with sympathomimetics using 2-deoxyglucose autoradiographic procedures. Rats were implanted with unilateral cannulae guides aimed for the nucleus accumbens. Following recovery, the animals were injected in the nucleus accumbens with either 15 nmoles of d-amphetamine sulfate or 50 nmoles of cocaine HCl. These doses were chosen on the basis of previous studies which revealed that they were effective in increasing locomotor behavior. Three minutes following nucleus accumbens injections the rats were administered 100 μ Ci/kg of [14 C]-2-deoxyglucose (2DG) through indwelling jugular catheters. Forty-five minutes following 2DG injections, the animals were prepared for autoradiographic analyses using standard procedures.

3. Modulation of Immune Function by the CNS

Numerous observations indicate that opiates can affect immune function. The presence of opiate receptors on leukocytes and endorphin production by these cells suggest a role for the opiate system in internal regulation of immune function. Direct *in vitro* effects of opiates on antibody production, lymphocyte proliferation induced by mitogens, and cytotoxic responses support this notion. Effects of opiates on the immune system *in vivo*, however, suggest that opiates may also act indirectly to modulate immune function. We have previously shown that chronic administration of morphine in mice alters T-cell functions involved in antibody production. Others have demonstrated morphine suppression of natural killer (NK) cell activity in rats and, moreover, have shown that intracerebroventricular (ICV) administration of morphine at a much lower dose produced a similar suppression of NK cell function, suggesting that this effect was mediated through the CNS.

In our present studies, we have attempted to localize the actions of opiates in suppressing immune function. Rats were implanted with cannulae guides aimed for the lateral ventricles or for various brain sites. One week following surgery, the animals received bilateral injections of morphine (5 μ g in 1 μ l saline) into the brain sites or unilateral injections (20-100 μ g) into the ventricles. Three hours following injection, the rats were sacrificed, spleens removed, and both NK cell activity and mitogen-stimulated lymphocyte proliferation were measured.

One region of the brain that appeared to be uniquely involved in modulating immune function following the direct application of morphine was the periaqueductal gray matter (PAG). We have recently attempted to further define the participation of the PAG in the regulation of immune function with other procedures as well. Rats were implanted with bipolar electrodes in various mesencephalic sites includ-

ing the PAG and reticular formation. Following recovery, the animals were stimulated through the electrodes for 10-15 minutes with intermittent stimulation. Following stimulation the rats were sacrificed, spleens removed, and both NK cell activity and mitogen-stimulated lymphocyte proliferation were measured.

4. The Effects of Phencyclidine on Nigrostriatal and Mesolimbic Functions

Phencyclidine (PCP) is a powerful psychotomimetic substance that produces psychopathological effects that mimic the primary symptoms of schizophrenia. Many of the effects of PCP have been thought to involve dopaminergic mechanisms. We have utilized a number of neurobiological techniques to ascertain the precise interactive effects of PCP with mesolimbic as well as nigrostriatal dopaminergic neurotransmission. In the first series of studies, rats were implanted with cannulae guides aimed for the terminal and cell body areas of the mesolimbic and nigrostriatal dopamine pathways. Rotational behavior was used to assess the interactive effects of PCP with the nigrostriatal dopamine system while locomotor activity was used to assess the effects of PCP on the mesolimbic dopamine system.

In order to further define the functional outputs of the nigrostriatal system that are activated by PCP, rats were implanted with unilateral cannulae guides aimed for the substantia nigra as well as with i.v. jugular catheters. Following recovery, the animals were injected in the substantia nigra with 25 nmoles of PCP. Three minutes later, the same rats were also injected i.v. with 100 μ CI/kg of 2DG. Forty-five minutes following administration of 2DG, the animals were sacrificed. The brains were prepared for autoradiographic analyses using standard procedures.

5. Kappa Opiate Receptor-Mediated Effects

A great deal of effort has been devoted to the development of non-addictive opiate analgesics. Originally, it had been hoped that certain benzomorphan analogs that interact selectively with kappa opiate receptors might have the desired characteristics to suit this need. Recently, a very selective kappa receptor agonist (U-50,488) became available which was subsequently resolved into (l) and (d) enantiomers. Preliminary findings indicate that the l-enantiomer is 4,000 times more potent than the d-enantiomer in kappa receptor binding assays, making the d-enantiomer an ideal substance to control for nonspecific U-50,488. In our initial series of studies, we evaluated and compared the effects of (l) and (d) U-50,488 on locomotor behavior, feeding, and drinking following intraventricular injections.

B. Major Findings

Acute injections of nicotine to rats lesioned unilaterally in the substantia nigra with 6-hydroxydopamine had little effect on rotational output during the first 30 minutes following injection. During the second 30 minute period following injection of 0.5 mg/kg of nicotine, however, a modest but significant increase in rotational behavior ipsilateral to the lesion was noted. Lower doses were ineffective in modifying this behavior. Injections of 0.5 mg/kg of nicotine in animals pretreated with amphetamine were found to potentiate rotational behavior ipsilateral to the lesion induced by amphetamine. Mecamylamine pretreatment prevented nico-

tine-induced rotational behavior. Chronic injections of nicotine induced rotational behavior that increased in intensity over days. These data suggest that nicotine induces a functional activation of the nigrostriatal dopamine system. Cytisine injections into the ventral tegmental area were found to increase locomotor output. This effect was antagonized by 6-OHDA lesions of the nucleus accumbens. Injections of cytisine into the VTA were also accompanied by increases in dopamine as well as DOPAC in the n. accumbens, while injections of cytisine into the n. accumbens had no effect on dopamine metabolites in this structure.

Unilateral microinfusions of kainic acid into the pedunculo-pontine nucleus increased the firing rate of dopaminergic neurons in the ipsilateral substantia nigra. Excitation was dose-related and occurred within seconds, indicating that the drug action was indirect. The kainate-induced excitation of dopaminergic neurons was prevented by intravenous administration of the centrally-acting nicotinic cholinergic antagonist mecamylamine. These results support the notion that cholinergic parikarya in the vicinity of the pedunculo-pontine tegmental nucleus innervate dopaminergic neurons in the substantia nigra zona compacta via nicotinic receptors. Furthermore, it appears that nicotine functionally activates both nigrostriatal as well as mesolimbic dopamine neurons in brain. It is suggested that the additive properties of nicotine, like other drugs of abuse, are mediated through an activation of central dopaminergic neurons.

Injections of amphetamine and cocaine were found to decrease metabolic activity in the head of the caudate nucleus, olfactory tubercle, cingulate cortex, as well as the nucleus accumbens ipsilateral to the injection. Significant increases in metabolic activity, however, were found in the ipsilateral ventral pallidus and the striatal fundus. The activity in some thalamic nuclei decreased ipsilateral to the injection. In the hindbrain, increases in activity were found in the zona reticulata and ipsilateral medial and lateral olivary complex. Combined with lesioning procedures, the 2DG methodology may allow a more comprehensive analysis of the functional outflow from behaviorally relevant neural systems.

Injections of 20-100 µg of morphine into the lateral ventricle produced a dose-dependent suppression of NK cell activity, suggesting that the effects of morphine on the immune system are probably mediated, at least in part, through the CNS. In subsequent studies, we have found that opiates act specifically in the periaqueductal gray matter (PAG) to produce suppression of two parameters of immunocompetence. Injections of morphine into the anterior hypothalamus, arcuate nucleus, medial amygdala, medial thalamus, and dorsal hippocampus had no significant effect on the parameters of immunocompetence examined when compared to uninjected controls. Injections of morphine into the PAG, however, produced a significant suppression of NK cell activity and T-cell proliferation in response to mitogen, when compared to saline-injected animals. These findings suggest that the central actions of opiates on immune function are mediated through the PAG. The precise neural mechanisms involved, however, remain to be elucidated. More recently, we have found that direct electrical stimulation of the PAG also compromises immune function in rats. It appears that this primitive core of the brain is a critical region for regulating the immune system.

Our findings have revealed that although PCP appears to produce behavioral effects indicative of DA activation, there is no evidence of direct dopamine involve-

ment. Injections of PCP into the nucleus accumbens (the terminal region of the mesolimbic dopamine system) produced locomotor excitation characteristic of dopamine receptor activation or dopamine release. This behavior, however, was not modified by 6-OHDA lesions of the nucleus accumbens or by haloperidol, indicating that PCP was producing behavioral effects independent of dopamine function.

PCP appeared to have little direct effect on striatal function although injections of PCP into the substantia nigra also produced behaviors indicating alteration of the DA nigrostriatal system. Further analyses using 2DG as well as selective lesioning techniques, however, have revealed that the effects of PCP at the levels of the substantia nigra are not mediated through the ascending dopamine nigrostriatal pathways, but involve systems projecting caudally to the pontine reticular structures.

While PCP does not appear to produce the behavioral effects through dopamine pathways, there is recent evidence suggesting that some of the effects of PCP may be mediated through an excitatory amino acid receptor. It has been found that PCP is a noncompetitive antagonist at the NMDA excitatory amino acid receptor. We have recently found that AP5 (a phosphoric acid analog which is a competitive antagonist at the NMDA receptor) produces behavioral effects in rats which are identical to those produced by PCP.

In a 75-minute test of motor activity, repeated administration of different doses of d-U-50,488 (10, 25, 50, 100 nmol) had no effect on rats' horizontal activity; however, 50 nmol did significantly decrease vertical activity ($p < .05$). Repeated administration of different doses of l-U-50,488 (10, 25, 50, 100 nmol) significantly increased horizontal activity at the 25 ($p < .05$) and 100 nmol ($p < .01$) doses. The effects of 100 nmol of l-U-50,488 on horizontal activity was antagonized by 5.0 mg/kg (i.p.) naloxone.

In a separate study, the acute administration of 100 nmoles of d-U-50,488 produced a significant depression of locomotor activity which was not antagonized by naloxone. The l-enantiomer also produced a modest but not statistically significant depression of locomotor output which was surprisingly enhanced by naloxone. The initial depressant effects of the l-U-50,488 were followed by locomotor excitation which was antagonized by naloxone. The d-enantiomer again had no excitatory effect on locomotor output.

Increases in the intake of a highly palatable food were seen in non-food-deprived animals after ICV injections of l-U-50,488 (25 & 100 nmoles). The increase in food intake induced by l-U-50,488 (100 nmoles) was antagonized by 1.0 mg/kg (i.p.) naloxone. The d-enantiomer had no effect on food intake.

Depression of water intake was seen in 21-hour water-deprived animals following repeated administration of different doses of l-U-50,488 (25 & 100 nmoles). Repeated administration of different doses of the d-enantiomer had no effect on deprivation-induced drinking.

These findings clearly demonstrate that some of the centrally mediated behavioral effects of the kappa agonist U-50,488 are stereospecific. The l-enantiomer enhanced locomotor activity, increased food intake, and decreased water intake.

The d-enantiomer did not produce similar effects. These compounds will undoubtedly prove useful in analyzing the specific effects of kappa receptor-mediated behaviors.

Significance to Biomedical Research and the Program of the Institute

Since opiate alkaloids produce some of the most profound behavioral and physiological effects, endogenous opiates (which are mimicked by the alkaloids) must serve an important role in regulating emotions as well as physiological and sensory processes. The use of our newly developed autoradiographic procedures, which allow a measurement of ongoing activity in these systems, may reveal the functional significance of opiate pathways in brain.

Since nicotine is one of the most abused substances in society, understanding its neuronal mechanisms of action will aid in understanding the abuse properties of this substance.

Phencyclidine is also an increasingly abused substance. Furthermore, phencyclidine produces effects in man very similar to some of the primary symptoms of schizophrenia. For these reasons, it is valuable to understand the mechanisms of action of this class of compounds.

It has been suggested that the cholinergic system also plays an important role in mental disorders. It is therefore necessary to understand the functions of this system in brain, and to analyze its interactive effects with other neurotransmitter systems such as dopamine.

Besides endorphins, the brain contains numerous other peptides which undoubtedly serve important regulatory functions. It is important to identify the distribution of binding sites for other substances in brain and to analyze their physiological and behavioral effects with micro-injection mapping techniques. Alterations in the activity of these systems may underlie a number of neurological and psychiatric disorders.

Proposed Course of Project

1. The in vivo autoradiographic technique will continue to be used to define endorphinergic circuitry activated by various behavioral and physiological manipulations.
2. Microdialysis procedures will be introduced and utilized to assess the activity of catecholamine systems in rat brain following the introduction of neuropeptides and other psychoactive compounds.
3. Attempts will be made to assess the functional activity of catecholamine systems during various behavioral states in the unanesthetized, freely-moving rat.
4. Functional metabolic activity of specific brain circuits will be assessed during various behaviors and following focal injections of various drugs using 2-DG procedures.
5. The evaluation of behavioral and physiological effects of neuropeptides will continue.

6. The specific brain circuits regulating immune function will be defined using electrical stimulation and microinjection procedures.

7. Further attempts will be made to localize opiate receptor subtypes on serotonergic, noradrenergic, and dopaminergic systems.

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Ostrowski, N.L., Pert, C.B. and Pert, A.: Visualization of opiate receptors in vivo. In Leslie, F. (Ed.): Receptor Localization: Ligand Autoradiography. New York, Alan Liss Inc., in press.

Pert, A. and Clarke, P.B.S.: Nicotinic modulation of dopaminergic neurotransmission: functional implications. In Martin, W.R., Van Loon, G.R., Davis, D.L., and Iwamoto (Eds.): Tobacco Smoking and Health: A Neurobiological Approach. New York, Plenum Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00400-05 BP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protein Phosphorylation in Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Jitendra Patel, Visiting Associate, Unit on Neurochemistry, BPB, NIMH

Sheela Vyas, BPB, NIMH; Anne-Marie O'Carroll, LCS, NIMH; Douglas Kligman, LCB, NIMH; Savella Detera, CNB, NIMH; Peter Fishman, DMNB, NINCDS; John Bishop, ET, NINCDS

COOPERATING UNITS (if any)

CNB, LCS, LCB, NIMH; DMNB, ET, NINCDS

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Neurochemistry

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.4

PROFESSIONAL:

1.2

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have extended our characterization of the major protein kinase C substrates of 87,000 dalton (Mr 87). We have purified this phosphoprotein to homogeneity. Purified Mr 87 was used to elucidate its amino acid composition, site of phosphorylation and partial amino acid sequence. Polyclonal antibodies to Mr 87 were raised and were used to perform immunolocalization of this phosphoprotein. Cloning of Mr 87 and other studies attempting to elucidate the function of MR 87 are in progress.

Our previous work had demonstrated that protein kinase C is involved in the regulation of receptor activity and neurotransmitter release. This work was further extended.

Further characterization and isolation of the 94,000 dalton cyclic AMP-dependent protein kinase substrate was performed.

PROJECT DESCRIPTION:

Objectives: The objective of this laboratory is to elucidate the chain of biochemical events, in particular protein phosphorylation, that is triggered ensuing receptor activation.

A number of different approaches are adopted in such investigations and they include:

1. Identification of particular protein kinase and phosphoproteins involved in specific receptor-triggered response either at the cellular level (e.g., receptor desensitization, stimulus-secretion coupling) or the behavioral level (e.g., kindling).

Methods Employed: protein purification, tissue culture, enzyme kinetics

Major Findings: A number of attempts have been made to identify the protein substrates that mediate the effect of protein kinase C. One such substrate that appears to be phosphorylated in a wide variety of tissues, including the brain, is a protein with apparent molecular weight of 87,000 daltons. In an attempt to elucidate the function of this phosphoprotein, we have purified Mr 87K phosphoprotein to apparent chemical homogeneity and have performed the following (in collaboration with Drs. Kligman and Detera).

1. We have raised highly specific polyclonal antibodies to Mr 87K.
2. Immunocytochemical and immunohistochemical localization of Mr 87K.
3. Using a gt-11 expression library from rat brain, we have identified a number of cDNA clones that selectively hybridize with the Mr 87L antibodies. The nucleotide sequence of the cDNA inserts has been determined.
4. We have determined partial amino acid sequence of a number of purified tryptic fragments of Mr 87K. Based on this sequence oligonucleotides have been synthesized and are now being used to screen gt-11 cDNA library from rat brain. A number of positively hybridizing clones have been identified and are now being characterized.

The role of protein kinase in stimulus-secretion coupling in endocrine cells was investigated using CRF-stimulated endorphin release from AtT-20 cells as a model system. A procedure to deplete AtT-20 cells of protein kinase C was elucidated and characterized. Using these cells, the role of protein kinase C in the receptor-activated neurotransmitter secretion and biosynthesis was investigated. This work was performed by Dr. Vyas in collaboration with Mr. Bishop and will shortly be submitted for publication in the Journal of Neurochemistry. A similar experimental approach was also undertaken in collaboration with Dr. Fishman, to elucidate the role of protein kinase C in the regulation of beta-adrenergic receptor-coupled adenylate cyclase activity. This work has now been published (Fishman et al., Biochem. Biophys. Res. Commun., 144:620-627, 1987).

In an attempt to elucidate the role of protein phosphorylation in the "kindling" process, the modulation of kinase activities were investigated in the hippocampus of lidocaine-kindled rats. A significant elevation in cyclic AMP-dependent protein kinase and protein kinase C activities were observed in kindled animals. Unlike protein kinase C, the elevation of cyclic AMP-dependent protein kinase activity did not appear to be associated with the seizures that the animals experience during the kindling process.

Dr. O'Carroll has continued attempts to define a protocol to purify a 94,000 dalton cyclic AMP-dependent protein kinase substrate. Recently, polyclonal antibodies to 94,000 dalton phosphoprotein were raised and these are now being characterized.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE:

The study of protein phosphorylation is of vital importance in the quest to better understand how the neurotransmitters mediate their action. Such an understanding is the necessary prerequisite for the appreciation of the biological basis of normal and abnormal behavior.

PROPOSED COURSE OF PROJECT:

These studies are expected to continue for the next several years.

PUBLICATIONS:

Patel, J. and Kassis, S.: Concanavin A prevents phorbol-mediated redistribution of protein kinase C and beta-adrenergic receptors in rat glioma C6 cells. Biochem. Biophys. Res. Commun. 144: 1265-1272, 1987.

Patel, J. and Kligman, D.: Purification and characterization of an Mr 87,000 (Mr 87) protein kinase C substrate from rat brain. J. Biol. Chem. 1987, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01831-11 BP

PERIOD COVERED

October 1, 1986 - September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Basic and Clinical Studies of Neuronal and Glial Enolases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Paul J. Marangos, Ph.D. Chief, Unit on Neurochemistry, BPB, NIMH

J.M. Polak Chairperson, Histochemistry Dept., Royal Medical School, London
A.G. Pearse Professor Emeritus, Royal Medical School, London
D. Schmechel Assoc. Professor, Neurology, Duke University
E. Ginns Neurologist, NSB, NIMH
B. Martin Biochemist, NSB, NIMH
D. Van Lubitz Physiologist, Georgetown Univ. Medical School

COOPERATING UNITS (if any)

Royal Med. School, London; Hoffmann La Roche; Duke U.; Vanderbilt U.; U. of Texas; U. of Virginia; UCLA Med. School; U. of Ulm, Germany; Georgetown Med. School; NSB, NIMH; Children's Hosp., Phila.; Johns Hopkins U.; Finsen Inst., Copenhagen.

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Neurochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.4

PROFESSIONAL:

0.5

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The basic neurobiology and potential clinical utilization of neuron specific enolase (NSE) has been a long-standing interest in our laboratory. During the past year, studies have begun to focus on the molecular biology of NSE in an effort to use the brain enolase isoenzyme system as a model to study neuron specific gene expression. We now have cdna probes for both NSE and non-neuronal enolase (NNE). The probes have been characterized and in situ hybridization studies have been performed in human brain. Our cdna probe for NSE only labels neurons whereas the probe for NNE preferentially labels glial cells. Studies performed using northern blots in both rat and guinea pig brain reveal that the mRNA for NSE is substantially larger than that for NNE. There is approximately a 75% homology within the coding region for human NNE and NSE (some 1700 base pairs). The 3'non-coding region is much larger for NSE and has very little homology to that of NNE, a fact we made use of to generate specific probes. Studies are in progress regarding the NNE to NSE switch that occurs during neural differentiation.

Studies are also in progress to determine the effect of cerebral ischemia on brain NSE levels in gerbil brain. In these studies we will attempt to demonstrate that NSE levels can be used as an index of neural damage post ischemia. The effect of various protective agents such as adenosine receptor agonists will also be assessed. Clinical studies assessing NSE levels in both human CSF and serum consequent to stroke are also in progress. The goal here is to determine whether NSE levels are correlated with the degree of post-ischemic neurologic damage.

Other Professional Personnel

S. Cohen	Neurologist	Georgetown Med. School
M. Usetegi	Director of Diagnostic Research	Hoffmann La Roche, Nutley, NJ
P. Zeltzer	Assoc. Prof., Pediatric Oncology	U. of Texas, San Antonio
D. Johnson	Associate Professor, Oncology	Vanderbilt Med. School
A. Greco	Assoc. Professor, Oncology	Vanderbilt Med. School
L. Rubinstein	Chairman, Dept. of Pathology	University of Virginia
R. Seeger	Pediatric Oncologist	UCLA Medical School
A. Evans	Chief, Pediatric Oncology	Children's Hosp., Phila.
V. Balasubramanian	Assistant Professor	Div. of Nuclear Med., Johns Hopkins University
A. Pedersen	Clinical Oncologist	Finsen Institute, Copenhagen, Denmark
K. Schilling	Cell Biologist	Univ. of Ulm, W. Germany
C. Pilgrim	Chairman, Dept. of Anatomy	Univ. of Ulm, W. Germany
D.T. Nakajima	Visiting Fellow	BPB, NIMH

Project DescriptionA. Objectives

The central theme of the Unit on Neurochemistry continues to be the characterization and potential clinical application of brain proteins. In this regard, our studies over the past decade have focused on both brain membrane proteins (neurotransmitter receptors) and on soluble neuron specific proteins such as the neuron specific enolase (NSE) and the glial specific protein S-100. The importance of cell-specific proteins is difficult to overstate, since it is clear that they probably are intimately involved in the specific differentiated functions of their parent cell. The characterization of such proteins, therefore, provides a useful approach to the study of neural physiology. The potential clinical applications of such proteins is also obvious, since antisera to these can serve as specific probes for the parent cell type.

Work done in our laboratory during the past decade has established that NSE is, in fact, specific to neurons and neuroendocrine cells, making it a highly useful marker for both cell types. We have also shown that NSE or the gamma enolase subunit, only appears in differentiated neurons. This makes it a highly useful marker for neural differentiation. We have also, at the clinical level, shown that NSE levels in various biological fluids can be of diagnostic importance in stroke patients and in two neuroendocrine cancers, small cell lung cancer, and pediatric neuroblastoma. Our studies concerning both the lung cancer and neuroblastoma patients have now been confirmed in several other laboratories and serum NSE assays are now becoming a part of clinical work-ups in both of these neuroendocrine cancers. The clinical information obtained from serum NSE levels has been shown to be highly useful for diagnosis, charting the clinical course of the illness, and determining the response to chemotherapy of individual patients. Our radioimmunoassay has become the standard to which other assays are compared, and several cancer diagnostic facilities are now using it to routinely screen patients.

Our major objectives in the NSE studies are two-fold, the first being to gain a better understanding of the role of NSE in neural physiology; i.e., why does the neuron require a unique form of this glycolytic enzyme? The second is to utilize the NSE methodology in neurologic and psychiatric patient populations. For the short term, we are now focusing on the utilization of serum and CSF NSE levels in stroke patients as an index of neurologic damage and prognosis.

B. Methods Employed

Gel electrophoresis, chromatography, northern, southern and western blotting, radioimmunoassay, immunocytochemistry, in situ hybridization, isoelectric focusing, and enzyme assays.

C. Major Findings

During the past year, the Unit has expended a major portion of time trying to incorporate a molecular biological component into the laboratory. This has involved the recruitment of a Fogarty Fellow, Dr. Takashi Nakajima, and the purchase of some equipment. Dr. Nakajima spent several months learning techniques related to RNA isolation, northern blotting, and cDNA probe processing in the laboratory of Dr. Edward Ginns, one of our major collaborators in these studies. We now have these procedures ongoing in our own laboratory, although the experiments using radioactive

tective effect on post-ischemic brain damage. The protective agents are adenosine receptor agonists such as cyclohexyladenosine. These studies utilizing NSE as an index of post-ischemic brain damage, therefore, interdigitate quite well with the adenosine receptor studies that are currently in progress in our laboratory. We have succeeded in working out the radioimmunoassay for gerbil NSE and are currently preparing our first group of animals where we will look at NSE levels in seven different brain areas at times ranging up to seven days post ischemia. We have plans for following up these experiments with immunocytochemical analysis where neural staining patterns with our anti-NSE serum will be analyzed.

In collaboration with Dr. Stanley Cohen at Georgetown, clinical studies are also in the planning stages concerning serum and CSF NSE levels in stroke patients. Previous work in our laboratory has documented an elevation in CSF NSE levels consequent to stroke. In the present study, we seek to extend these observations and attempt to determine whether a correlation exists between the severity of the stroke and the NSE level in CSF. We also will look at serum in the study in the hope that we will see elevated NSE levels. If this works out, it will make the application of the NSE methodology much more feasible in stroke patients.

To summarize, the past year has been one in which NSE-related studies have been refocused at both the basic and clinical levels. We are rekindling our basic studies, which will now focus on molecular biology and the mechanism of the alpha to gamma developmental switch process. Our efforts in the basic science of NSE have been very few in the past five years, with the major focus having been the clinical applications of NSE to neuroendocrine cancers (lung cancer and neuroblastoma), as mentioned in previous annual reports. In the clinical area, we intend to put more emphasis on neurologic and psychiatric disorders such as stroke and schizophrenia. We are currently planning not only to assess NSE levels in patient populations, but we would also like to be able to measure antibodies to NSE in patient sera. In this regard, we are in the process of developing an ELISA for NSE and hope to have it operational within several months. This will enable us to determine more of a biologic record on a patient related to neural tissue degeneration rather than having to rely on catching an elevation in actual NSE levels that is probably closely correlated temporarily with tissue destruction.

D. Significance to Biomedical Research and the Program of the Institute

The enolase isoenzyme system in brain represents an ideal system for studying neural differentiation. With NSE, we have a means of assessing neural function both chemically (RIA) and histologically (immunocytochemistry). Utilization of this unique methodology should greatly facilitate our understanding of neural development and function. Clinically, the NSE methodology has been shown to be of considerable utility for neuroendocrine cancers, with the potential also existing for its utilization in neurologic and psychiatric disorders. The major change of the IRP is to engage in novel neuroscience research that has the potential for increasing our understanding of brain function in both health and disease. It is clear that our work over the past decade closely parallels these goals.

E. Proposed Course of the Project

The studies will likely continue for at least the next two years.

PUBLICATIONS

Polak, J.M. and Marangos, P.J.: Neuron specific enolase, a marker for neuroendocrine cells. In Falkner, S., Hakanson, R. and Sundler, F. (Eds.): Evolution and Tumor Pathology of the Neuroendocrine System. Amsterdam, Elsevier, 1985, pp. 433-480.

Marangos, P.J. and Schmechel, D.R.: Neuron specific enolase, a clinically useful marker for neurons and neuroendocrine cells. Ann. Rev. Neurosci. 10: 269-295, 1987.

Giannone, L., Johnson, D.H., Grosh, W.W., Davis, B.W., Marangos, P.J., and Greco, F.A.: Serum neuron specific enolase in metastatic Merkel cell tumors. Med. Pediatr. Oncol., in press.

Schmechel, D.R., Marangos, P.J., Martin, B. and Ginns, E.: Localization of neuron specific enolase (NSE) mRNA in human brain. Neurosci. Lett., in press.

Marangos, P.J.: Neuron specific enolase, a neural and neuroendocrine protein. In Marangos, P.J., Campbell, I.C. and Cohen, R. (Eds.): Neurobiological Research, Vol. II. New York, Academic Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01833-07 BP

PERIOD COVERED

October 1, 1986 - September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Adenosine Receptors in the CNS

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Paul J. Marangos, Ph.D.	Chief, Unit on Neurochemistry,	BPB, NIMH
J. Patel	Biochemist,	BPB NIMH
R. M. Post	Chief	BPB NIMH
S. Cohen	Neurologist	Georgetown Univ.
Dag Von Lubitz	Neurologist	Georgetown Univ.
N. Sperekalis	Chairman, Dept. of Physiology	Univ. Cincinnati
K. Jacobsen	Staff Fellow	LBC, NIADD

COOPERATING UNITS (if any)

BPB, CNB, NIMH; LBC, NIADD; CP, NCI; U. of Cincinnati, Georgetown Univ.

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Neurochemistry

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

0.6

OTHER:

1.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The pre-clinical evaluation of both adenosine receptor and adenosine uptake site continue to occupy a major portion of the Unit's resources. We have described the complete anatomical distribution of the adenosine uptake site using [³H]dipyridamole autoradiography and shown by these binding experiments that there are multiple populations of brain adenosine uptake sites. Studies measuring adenosine uptake in synaptoneurosomes support the binding and autoradiography. More evidence has been gathered indicating that carbamazepine is acting as an adenosine antagonist. Xanthine-induced seizures have been studied using the new A₁-specific antagonist, XAC. Evidence was generated showing that XAC-induced seizures differ from those induced by caffeine in that they are not blocked by RO-15-1788, a benzodiazepine receptor blocker. We have also shown increased adenosine uptake sites in drug resistant human breast cancer cell cultures, suggesting that the adenosine uptake site may be involved in the phenomenon of multi-drug resistance.

Recent studies have focused on the role of adenosine agonists as protective agents during cerebral ischemia. The post-ischemic neuropathy is being characterized as it relates to adenosine, glutamate, and benzodiazepine status at both the binding and autoradiographic level. These studies will hopefully assess the potential utility of adenosine agonists in the treatment of stroke patients.

Other Professional Personnel:

P. F. Morgan	Fogarty Visiting Fellow	BPB, NIMH
J. Deckert	Guest Researcher	BPB, NIMH
J-L. Daval	Guest Researcher	BPB, NIMH
T. Nakajima	Fogarty Visiting Fellow	BPB, NIMH
S.R.B. Weiss	Staff Fellow	BPB, NIMH
T. Insel	Staff Fellow	CNB, NIMH
K. Jacobson	Staff Fellow	LBC, NIADD
R. Fine	Oncologist	CP, NCI

I. Project Description

A. Objectives

The characterization of specific receptor systems in the CNS is now recognized as a desirable first step in pre-clinical neuropharmacologic research. The rationale is that the elucidation of such systems will greatly facilitate the development of specific pharmacologic agents that interact with only the receptor system in question. Specificity of interaction is of obvious importance for the development of new drugs that will have a minimum of side effects. The radioreceptor binding methodology has proven to be of great utility in the discovery of new neurotransmitter and neuromodulator receptor systems in brain and can be highly useful in pre-clinical studies. We have, for the past ten years, applied this approach to the study of the benzodiazepine, GABA, and adenosine receptor systems and have, in the process, increased our understanding of the basic biology of each as well as the potential for pharmacologic intervention.

A major focus of our laboratory has centered around the mechanisms involved in the depression of neural activity. In this regard, both the benzodiazepine and adenosine systems play major roles. Since an endogenous ligand for the benzodiazepine receptor remains illusive, it is difficult to postulate an actual physiologic role for the benzodiazepine receptor. It is, however, quite clear that this is not the case as regards the adenosine system, and adenosine is, in fact, now widely conceptualized as being the brain's own natural depressant or sedative. Since the importance of the benzodiazepine receptor as an agent of *in vivo* physiologic significance is open to question (due to the lack of an endogenous ligand), we have become increasingly involved with the adenosine system as an object of study as regards the modulation of CNS arousal states. There is now ample evidence that adenosine is a major neuromodulator in brain having marked effects on cyclic AMP levels, neurotransmitter release, calcium fluxes, neuronal firing, and behavior. It is also clear that adenosine serves as the endogenous ligand, so all the elements of the system are at least identified. Also, the types of behaviors mediated by this system (arousal, sedation, convulsions, and post-ischemic protective effects) make it a good candidate for the development of new drugs of potential relevance to psychiatry and neurology.

B. Methods Employed

Scintillation counting, autoradiography, radioreceptor assays, tissue extraction, animal surgery, and chromatography.

C. Results

Our laboratory is virtually the only one in the world actively engaged in studies of both the adenosine receptor and the adenosine uptake site. We were the first to demonstrate that brain adenosine uptake sites could be labeled using [³H]-nitrobenzylthioinosine (NBI) and proceeded to characterize this site showing that it was totally distinct pharmacologically from the adenosine receptor. During the past year, we have worked further with a new ligand probe for the adenosine uptake site which we had custom labeled. This probe is [³H]dipyridamole (DPR), which we synthesized because we suspected that [³H]NBI was not recognizing all of the adenosine uptake sites present in brain.

Using [³H]DPR, we have shown by three different approaches that this ligand binds to several-fold more sites in the brain than does [³H]NBI, and have, in the

process been able to put forth the postulation that heterogeneity exists in brain adenosine uptake sites. In binding studies, we consistently show a higher number of binding sites for [^3H]DPR compared to [^3H]NBI (2- to 4-fold) and the inhibition of [^3H]DPR binding by unlabeled NBI is distinctly biphasic in nature. These results clearly suggest that an NBI-sensitive and an NBI-insensitive site is present. We have shown that this is the case in guinea pig, dog, and human brain.

In autoradiographic studies, Dr. Deckert has shown that [^3H]DPR is able to label more sites in cerebellum, hippocampus, and the various ependymal membranes lining the ventricles. Also, unlabeled NBI is not as effective in displacing [^3H]DPR as is unlabeled DPR, further supporting the binding studies as regards adenosine uptake site heterogeneity. Dr. Deckert has also shown a substantially better co-localization of adenosine uptake sites with the adenosine receptor when he uses [^3H]DPR as the ligand probe. This has been very important, since it is a major criterion for the identification of adenosinergic neurons; i.e., the co-localization of both the receptor and the uptake site.

Dr. Philip Morgan has shown, using actual measurements of [^3H]adenosine into synaptoneurosome, that DPR has different properties from NBI. Dr. Morgan's studies show a biphasic inhibition of [^3H]adenosine uptake by NBI and a monophasic inhibition of DPR. These elegant studies again totally complement the binding and autoradiographic studies in that they strongly suggest the existence of NBI-sensitive and NBI-insensitive sites and, consequently, adenosine uptake site multiplicity.

All of the above mentioned studies are currently in press in various journals in a total of five manuscripts.

In an effort to further extend our understanding of the adenosine uptake site, we undertook a study of the ontogenic development of adenosine uptake sites and how they relate to the receptor to the cyclase. Here, Dr. Morgan has shown that in rat brain the uptake site is high from very early in development, followed by the appearance of the receptor and the coupling mechanism. We are currently extending these studies to look at the development of adenosine uptake site subtypes in guinea pig brain. Preliminary results show that the ratio of [^3H]DPR to [^3H]NBI sites increases during guinea pig brain development.

In all of the above studies, the goal is at some point to be able to develop the ability to selectively modulate each sub-population of adenosine uptake sites in brain. Such selective drugs may prove to have beneficial sedative, anxiolytic, or anti-ischemic properties and would be highly specific.

We have begun to shift our focus this past year toward more clinical applications as regards the adenosine system. Basically, this has been in three areas which are: 1) adenosine as a protective agent against brain ischemia; 2) multi-drug resistance of cancer cells and the adenosine uptake site; and 3) adenosine as an anticonvulsant and its relationship to carbamazepine.

We have recently begun a collaboration with Drs. Von Lubitz and Cohen from Georgetown University dealing with the effects of adenosine agonists on post-ischemic neurologic damage. These investigators have shown dramatic protective effects of cyclohexyladenosine (CHA) when given up to 30 minutes after ischemia in gerbils.

CHA-treated animals (>90%) survive ischemia, whereas >80% of untreated animals die within 8 hours after the ischemia (30 minutes of carotid artery occlusion). We are currently investigating the pharmacology of this effect as well as the neuropharmacologic, neurochemical, and neuroanatomic consequences of ischemia, comparing both the treated and untreated animals. Our hypothesis is that adenosine agonist treatment prevents glutamate release post-ischemia, thereby protecting these neurons from the excitotoxic syndrome. We hope to show that CHA and adenosine uptake blocker treatment actually does preserve neural function as judged by adenosine and glutamate receptor autoradiography. We will also attempt to show blockade of the effect by adenosine antagonists and to compare the potency of adenosine agonist effects with those of glutamate antagonists. These studies will constitute a major research effort in our laboratory and it is expected that they will be of major clinical relevance.

In collaboration with Dr. Robert Fine from NCI, Dr. Morgan has just completed a rather interesting study which has shown, at the level of both [³H]NBI and [³H]DPR binding, as well as by direct [³H]adenosine uptake, that cultured human breast cancer cells which are resistant to chemotherapeutic drugs manifest a dramatic increase in adenosine transport. This is an intriguing finding which suggests that the tone of the adenosine transporter may be significant as it relates to the sensitivity of a cell towards chemotherapeutic drugs. This finding is currently being written up for publication with future studies planned to dissect out the potential significance of these observations. It would certainly be intriguing if we could demonstrate a reversal of drug resistance with adenosine uptake blockers.

Our long-term studies regarding the interaction of the anticonvulsant and antimanic agent carbamazepine with adenosine receptors have also continued during the past year and yielded some new and interesting results. We have shown, in collaboration with Drs. Post and Weiss, that the upregulation of adenosine receptors produced by carbamazepine is quite long-lasting and possibly irreversible, since it persists for at least eight weeks after carbamazepine withdrawal. We have also provided convincing biochemical evidence that carbamazepine acts as an adenosine antagonist in that it is more potent at lower temperatures as an inhibitor of adenosine agonist binding, and that its potency as an inhibitor of adenosine agonist binding increases in the presence of a guanosine triphosphate analog. Both the pharmacologic and the biochemical study are in press and both are consistent with an antagonist interaction of carbamazepine with adenosine receptors. Future studies will focus on the mechanism of carbamazepine-induced adenosine receptor upregulation and its brain regional aspects in comparison with the effect of chronic caffeine. In this regard, we have currently embarked on a major effort to establish a cell culture facility within the Unit. Although we have been severely hampered by space limitations, we have managed to set up an incubator and a sterile hood in another laboratory, and have begun to grow some transformed cells such as rat PC-12 and human LAN-1 neuroblastomas. We are now attempting to grow primary rat neural cultures. Our goal in these efforts is to study the effects of chronic and acute carbamazepine and caffeine treatment at the molecular level and ask questions such as whether the adenosine receptor is being phosphorylated and whether modulators such as phorbol esters can affect carbamazepine-induced adenosine receptor upregulation. The cell culture approach offers distinct advantages for mechanistic studies in that variables can be more effectively controlled.

D. Significance to Biomedical Research and the Program of the Institute

There is a great need for new and more effective psychotherapeutic drugs. It is only through an increased understanding of brain function that we can hope to determine the potential sites for drug intervention. Characterizing defined brain systems that mediate important neural mechanisms is therefore an important preclinical research strategy. In this regard, both the adenosine and benzodiazepine systems are good targets for increasing our conceptions of the mechanisms involved in seizures, sedation, and anxiety, all of which are of clinical importance.

E. Proposed Course of the Project

Studies should continue for several years.

PUBLICATIONS

Bisserbe, J.C., Deckert, J. and Marangos, P.J.: Autoradiographic distribution of [³H]dipyridamole binding sites in guinea pig brain. Neurosci. Lett. 66: 341-345, 1986.

Deckert, J., Bisserbe, J.-C., and Marangos, P.J.: [³H]dipyridamole and [³H]nitrobenzylthioinosine binding sites in guinea pig brain: a comparison. Pflugers Arch. Eur. J. Physiol. 407 (Suppl 5): 29, 1986.

Deckert, J. and Marangos, P.J.: Hormonal interactions with benzodiazepine binding sites in vitro. Life Sci. 39: 675-683, 1986.

Marangos, P.J.: Biochemical and pharmacologic properties of brain adenosine receptors and uptake sites. Pflugers Arch. Eur. J. Physiol. 407 (Suppl 5), 1986.

Marangos, P.J.: Calcium antagonists and the brain adenosine system. In Shagass, C., Josiassen, R.C., Bridger, W.H., Weiss, K.J., Stoff, D. and Simpson, G.M. (Eds.): Biological Psychiatry, 1985 (Developments in Psychiatry, Vol. 7). Amsterdam, Elsevier, 1986, pp. 308-311.

Marangos, P.J., Deckert, J., and Bisserbe, J.C.: Central sites of adenosine action and their interaction with various drugs. In Gerlach, E. and Becker, B.F. (Eds.): Topics and Perspectives in Adenosine Research, 1986, pp. 1-634.

Tamborska, E., Insel, T., and Marangos, P.J.: "Peripheral" and "central" type benzodiazepine receptors in Maudsley rats. Eur. J. Pharmacol. 126: 281-289, 1986.

Marangos, P.J. and Deckert, J.: [³H]dipyridamole binding to guinea pig brain membranes, possible heterogeneity of central adenosine uptake sites. J. Neurochem. 48: 1231-1237, 1987.

Marangos, P.J., Patel, J., Smith, K., and Post, R.M.: Adenosine antagonist properties of carbamazepine. Epilepsia 28: 387-394, 1987.

Deckert, J., Bisserbe, J.-C., and Marangos, P.J.: Quantitative [³H]dipyridamole autoradiography: evidence for adenosine transporter heterogeneity in guinea pig brain. Naunyn-Schmiedeberg's Arch. Pharmacol., in press.

- Deckert, J., Estal, L.B., Marangos, P.J. and Cooper, S.J.: CGS-8216 treatment decreases central type benzodiazepine receptors in rat brain. Eur. J. Pharmacol., in press.
- Klein, E., Marangos, P.J., Montgomery, P., Bacher, J., and Uhde, T.W.: Adenosine receptor alterations in nervous pointer dogs, a preliminary report. Clin. Neuropharmacol., in press.
- Marangos, P.J., Campbell, I.C. and Cohen, R.M. (Eds.): Neurobiological Research, Vol. II: Functional and Clinical Aspects of Neuronal and Glial Proteins. New York, Academic Press, in press.
- Marangos, P.J., Insel, T.R., Montgomery, P. and Tamborska, E.: Brain adenosine receptors in Maudsley reactive and non-reactive rats. Brain Res., in press.
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- Morgan, P.F. and Marangos, P.J.: Ontogenetic appearance of the adenosine receptor precedes N-protein coupling in rat forebrain. Develop. Brain Res., in press.
- Morgan, P.F. and Marangos, P.J.: Comparative aspects of nitrobenzylthioinosine and dipyridamole inhibition of adenosine accumulation in rat and guinea pig synaptoneuroosomes. Neurochem. International, in press.
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00450-13 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Rhythms in Affective Illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Sack	Chief, Inpatient Services	CPB/NIMH
Others:	W. Mendelson	Chief, Unit on Sleep Studies	CPB/NIMH
	W. Duncan	Research Psychologist	CPB/NIMH
	N. Rosenthal	Chief, Outpatient Services	CPB/NIMH
	R. Skwerer	Medical Staff Fellow	CPB/NIMH
	F. Jacobsen	Medical Staff Fellow	CPB/NIMH
	T. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In our present study we have been comparing the circadian system of depressed patients and normal controls under routine ward conditions and then repeating these measurements in conditions where the internal and external sources of masking have been controlled by holding diet, activity, posture, lighting and wakefulness constant.

Our preliminary data indicated that the temperature rhythm in patients with affective disorders is increased in both depression and mania compared to controls and the timing, or phase of the temperature is shifted to an abnormally early time in both phases of the illness. We have studied an additional 5 patients and 5 normal controls and the results of these experiments are being analyzed. We have confirmed our previous observation that nocturnal TSH secretion is decreased and the response to sleep deprivation is diminished in depression, in a new study of 34 predominantly unipolar patients. Thus, diminished nocturnal TSH secretion does not appear to be restricted to a particular diagnostic subgroup of depression but is a characteristic feature of this disorder.

In order to better understand the basis for the circadian and sleep dependent changes in TSH secretion, we have investigated the response of TSH to TRH administered intravenously in the morning and at night with subjects awake and asleep. Δ TSH was significantly higher when subjects were awake at night compared with asleep whereas circulating free and total thyroid hormones did not differ on the two conditions. These data suggest that lower TSH levels during sleep are due to changes in pituitary sensitivity to TRH which are central in origin, perhaps due to the inhibitory effect of one or more neuropeptides that affect TSH secretion.

Project Description:

The details of this project have been extensively described in annual report Z01 MH 00450-11 CP and will only be reviewed briefly here. Abnormalities in the circadian rhythms of hormones, neurotransmitters, and body temperature have been previously described in depressed patients compared with controls. Many of these observations have not been consistently replicated and this may have been due to artifacts related to diet, activity, sleep and posture. In addition apparent differences in circadian rhythms in patients and normals may occur secondary to changes in behavior rather than to a change in the properties of the intrinsic biological clock or clocks regulating these systems. In order to determine the basis for apparent differences in the circadian rhythms in patients with affective disorders we have measured the circadian rhythms in a number of physiological variables under our usual ward conditions and have repeated these measurements under conditions where activity, sleep, temperature, diet and wakefulness were held constant. An additional purpose has been to determine the relationship between disturbances in circadian rhythms and the antidepressant effects of sleep deprivation.

Methods:

The methods have been described in detail in Z01 MH 00450-11 CP.

Findings to Date:

As part of a collaborative study with the University of Heidelberg, we have studied 34 patients with major depression and 12 normal controls using a new method derived from our previous studies. TSH and prolactin levels were measured at 2 am at baseline and during a night of total sleep deprivation. TSH levels and the increase in TSH secretion with sleep deprivation were significantly lower in depressed patients compared with normal controls. Sleep deprivation increased TSH levels in all controls subjects but levels actually decreased in 1/3 of the depressed subjects. Although sleep deprivation significantly reduced the severity of depression in the patients, there was no correlation between clinical improvement and hormone levels at baseline or their change during sleep deprivation.

In order to further understand the basis for abnormal nocturnal TSH secretion in depressed patients, we studied the nocturnal responses of TSH to TRH in 9 healthy controls. Subjects underwent IV TRH challenge tests (500 mcg) on three conditions: awake in the morning, asleep at night, and awake at night. TRH responses were significantly greater at night when subjects were awake than on either of the other two conditions but free and total thyroid hormone concentrations did not differ. These data suggest that lower TSH levels during sleep may be due to inhibition of TSH responsiveness which is central in origin.

Significance to Biomedical Research:

1. The disturbances in temperature regulation and in the hypothalamic-pituitary-thyroid axis suggest that patients with depression may suffer from an underlying disturbance of metabolism and thermogenesis and that the symptoms of depression and mania may reflect a physiological adaptation to this metabolic disturbance. Additional metabolic studies are required to elaborate on this hypothesis.
2. Contrary to our hypothesis, there was no correlation between the clinical response to sleep deprivation and changes in the hypothalamic-pituitary-thyroid axis. However, we have shown that low nocturnal TSH and a diminished response to sleep deprivation is a characteristic

hormonal abnormality in depression and have developed a simple and reliable method for assessing nocturnal TSH levels in a clinical population.

3. In our most recent experiment we have found that pituitary sensitivity to TRH varies as a function of time of day and state of consciousness (asleep versus awake). Sleep inhibits TSH responses to TRH via a central mechanism. Several peptides and neurotransmitters have been shown to inhibit TSH and night, and excessive inhibition by one or more of these may be responsible for the abnormal HPT responses in depressed patients.

Proposed Course:

We intend to study additional subjects in order to elaborate on our preliminary findings and in particular to clarify the relationship between these circadian disturbances and improvement with sleep deprivation and other antidepressant therapies.

Publications

Sack, D.A., James, S.P., Scherer, M.A., Linnoila, M., Wehr, T.A.: The diurnal variation in MHPG is abolished but a variation in HVA persists under constant conditions. *Arch Gen Psychiatry in press.*

Sack, D.A., James, S.P., Rosenthal, N.E., Wehr, T.A.: Deficient nocturnal surge of TSH during sleep and sleep deprivation in rapid-cycling bipolar patients. *Psychiatry Research in press.*

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Studies of Insomnia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. B. Mendelson Chief, Section on Sleep Studies CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Section on Sleep Studies

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.3

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our work has shown insomniacs have some disturbance of perception. We have reported insomniacs have cognitive difficulties during the day. Their sleep is not different when compared to normals, but insomniacs report only half as much sleep as their normal controls. This suggests insomniacs have some misperception of their state of consciousness.

In an earlier study, we used meaningful (subject's name) and meaningless (electronic tone) stimuli to arouse the subject from different stages of sleep (just after lights out, stage II, stage IV, REM, after a movement time). Subjects were most difficult to arouse from REM and stage IV. The subjects were insomniacs and their matched controls.

In our current study, we are interested in the effects of hypnotics on this paradigm. Although the effects of hypnotic on subjective and objective measures of sleep is well documented for continuous sleep, little is known about their effects on sleep with forced awakenings. We found the hypnotic, flurazepam, raised the arousal threshold for all stages of sleep, but did not alter subjective measures of sleep when compared to placebo.

This project is no longer being pursued by the Branch and was terminated in March, 1987.

Project Description:

Insomniacs were screened for chronic insomnia, then given a Polysomnogram to rule out sleep apnea and other sleep disorders. The study consists of four nights (2 stimulus conditions each with placebo and drug).

Methods:

Ten insomniacs were given a PSG and then adapted for one night. The study consisted of four nights. Subjects were awakened from 5 different stages of sleep. The arousal stimuli were the subject's name repeated in a monotone voice for 2 minutes (maximum stimulus duration) or a continuous electronic tone. Subjects were given drug and placebo in both stimulus conditions. When the subjects were awakened, they were asked a series of questions about their sleep and how they felt.

Findings to Date:

The arousal threshold differs across the stages of sleep. This is consistent with previous studies on arousal threshold. The arousal threshold was greatest for stage IV and REM. They were greater for meaningless than for meaningful stimuli. Flurazepam increased the arousal thresholds in both stimulus conditions and decreased sleep latency, but it did not effect the subjects perceptions of estimated time asleep.

Significance to Biomedical Research:

These data suggest while hypnotics raise arousal thresholds and decrease sleep latency, they do not alter an insomniac's preception of his sleep.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02197-01 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Study of the Effects of Light and Triazolam on Delayed Sleep Phase Syndrome

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N. Rosenthal, Chief, Outpatient Services CPB/NIMH

Others: K. Kelly Medical Staff Fellow CPB/NIMH
 J. Vanderpool Medical Staff Fellow CPB/NIMH
 P. Schulz Social Worker CPB/NIMH
 T. Wehr Chief, Clinical Psychobiology Branch CPB/NIMH
 E. Souetre Visiting Fellow CPB/NIMH

COOPERATING UNITS (if any)

Richard Allen, Ph.D., Johns Hopkins Sleep Center, Upjohn Pharmaceutical Company

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

0.5

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Delayed sleep phase syndrome (DSPS) is a condition characterized by a delay in the timing of sleep onset and waking. Generally present from an early age, DSPS usually produces considerable difficulties for those who are required to function on a 9-to-5 schedule. Most subjects have tried unsuccessfully to correct their sleep-wake cycle many times. One type of currently available treatment, chronotherapy, involves delaying the timing of sleep onset further each day until it reaches the desired clock time. Although this is quite effective in some cases, it is inconvenient and the benefits are often transient.

The object of the present study is to recruit subjects who complain of DSPS, to determine the clinical and demographic characteristics of these individuals, and to try to correct their circadian rhythm abnormalities by judicious use of exposure to bright light and darkness, and by using the short-acting benzodiazepine, triazolam. Both of these strategies are derived from studies of circadian rhythms in animals, the timing of which is ordinarily influenced by the light-dark cycle. In recent years we have found that light of intensity greater than ordinary indoor lighting has a variety of effects on brain function. For example, it is capable of suppressing melatonin, reversing depression in seasonal affective disorder, altering norepinephrine and serotonin metabolism, and affecting resting metabolic rate and immune function. There is also evidence that bright light can influence the timing of circadian rhythms in humans.

We identified 93 subjects with DSPS and noted their reported patterns of sleeping and waking, as well as the difficulties encountered as a result of their sleep problem. We have studied sleep architecture in 11 cases and have not found patients to be suffering from another type of sleep disturbance. Three patients have been treated with both active (bright light in the morning plus dark goggles in the evening) and control (dimmer light in the morning and transparent goggles in the evening) light treatment conditions, and it appears that the active but not the control condition has a beneficial effect on the timing of sleeping and waking. However, the results are preliminary and more subjects will have to be run before a definitive statement can be made as to the value of bright light in the treatment of DSPS. We have yet to explore the value of triazolam in this condition.

Project Description and Methods:

A group of patients, who have difficulty going to sleep and waking up at conventional times, has previously been described. This condition, termed delayed sleep phase syndrome (DSPS), has been shown to cause significant unhappiness in some individuals, who are unable to get to work on time and suffer the social consequences of being on a different sleep-wake schedule from most other people. Typically these individuals have made many unsuccessful attempts to correct their abnormal sleep-wake cycle; indeed, it often appears to be rigidly set in place. A non-pharmacological treatment, called chronotherapy, in which the patient is advised to go to sleep at progressively later times each day until the desired sleep onset is reached, has proven successful in some individuals. However, it is an inconvenient treatment, involving several days of disrupted schedules and, furthermore, the benefits appear to be short-lived in some cases, who soon find their sleep onset time drifting progressively later again.

As our understanding of the non-visual, biological effects of light (particularly bright light) has expanded, we have become aware that light may well play a significant role in setting the timing of sleeping and waking in humans. If this is so, then perhaps bright light could be used therapeutically to help patients with DSPS entrain to a more normal sleep schedule. Recent studies in rats have shown that the short-acting benzodiazepine, triazolam, is capable of shifting the timing of free-running biological rhythms. It is conceivable that this effect of the drug could be put to use in helping patients with DSPS entrain to a desired sleep-wake schedule. In fact, an optimal solution might be to use triazolam to help patients to comply initially with an unusual sleep schedule and, later, to use bright light alone to maintain the new sleep schedule.

In the present study, we plan to recruit a population of patients with DSPS, who are motivated to have their sleep schedules altered; and who are willing to participate in a research study in order to do so. The initial phase of the study will involve describing the clinical population by means of specially prepared screening instruments, clinical interviews and standardized structured psychiatric interviews. Patients will need to meet certain predetermined inclusion criteria in order to be included in the study. They will then be given daily rating forms for recording their sleep pattern and be asked to wear a wristwatch-like activity monitor for two weeks, in order to corroborate prospectively their history of delayed sleep phase. Patients will be given a physical examination and a polysomnogram in order to rule out other sleep disorders that could interfere with our making an accurate diagnosis, or with our ability to make effective interventions.

After the initial phase of the study, subjects will be treated in a crossover design in which conditions of light and dark will be manipulated. Subjects will be assigned to two two-week treatments in random order: 1) two hours of bright (2500 lux) full-spectrum light in the morning, in conjunction with dark goggles, worn for several hours in the evening; and 2) two hours of dim (300 lux) light in the morning, in conjunction with transparent goggles, worn for several hours in the evening. We predict that the former condition will be active and will have the effect of bringing dawn and dusk earlier; the latter condition should be inactive and thus serve as a control. If the timing of light and dark are important for the entrainment of circadian rhythms in DSPS patients, then one would expect that the active treatment would help entrain subjects to earlier sleep onset and wake times.

Findings to Date:

Ninety-three subjects who responded to our initial publicity met criteria for DSPS. Of these 69 (74%) were women and 24 (26%) were men. Mean age of responders (\pm S.D.) was 34.4 ± 10.4 years; Mean age of onset of noticeable, abnormally delayed sleep onset was 10.1 ± 9.0 years; and patients reported that their sleep schedules became a problem at 15.0 ± 10.8 years.

Sixty-eight subjects (72%) reported that their DSPS interfered with work functioning to a moderate or marked degree; a similar degree of impairment in interpersonal relationships was reported by 52 subjects (56%). On average subjects reported going to bed at 1.25 a.m. on weekdays and 2.26 a.m. on weekends but noted that sleep onset occurred somewhat later (2.15 and 3.05 a.m. respectively). Corresponding average wake-up times for week and weekend days were 9.20 and 10.50 a.m. Most patients (86%) described the quality of their sleep as sound.

Polysomnograms were run on 11 subjects and no sleep abnormality, other than the shift in timing, was noted in 9 of these cases. Three subjects have been run through the two treatment conditions thus far. They have observed that the bright light in the morning, combined with the use of dark goggles in the evening, has induced a significant improvement in daily rhythms. It has been easier for them to fall asleep and wake up earlier, and they have felt more alert in the morning. Preliminary analysis of sleep latency testing, performed at 9.00 a.m. shows that patients have longer sleep latencies after the bright light than after the dim light treatment. This latter, control condition was not experienced as helpful in any way.

Significance to Biomedical Research:

If bright light should prove to be helpful in re-entraining the timing of daily rhythms in DSPS, this will add a new clinical application to its increasing number of potential uses, as well as provide us with further insights into the effects of light on circadian rhythms in humans. Besides helping individuals with chronic abnormalities in circadian rhythms, this information may be helpful in the treatment of the more common temporary shifts in circadian rhythms found in shift-workers and in jet-lag, both of which produce dysphoria and functional impairment.

Proposed Course:

We plan to continue to run DSPS patients through the crossover study to determine whether bright light can indeed induce a statistically and clinically significant effect on the entrainment of their daily rhythms. In those people who are only partially helped by bright light we plan to use the short-acting benzodiazepine, triazolam, to improve compliance and enhance the phase-shifting effects of bright light.

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Early Versus Late Partial Sleep Deprivation in the Treatment of Depression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Sack	Chief, Inpatient Services	CPB/NIMH
Others:	T. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH
	N. Rosenthal	Chief, Outpatient Services	CPB/NIMH
	W. Mendelson	Chief, Unit on Sleep Studies	CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Total sleep deprivation, partial sleep deprivation, and shifting the time of the sleep period several hours earlier than usual can improve depression and can induce mania. Also, recovery sleep after these interventions can induce depression. Thus, changes in the timing and duration of sleep could trigger episodes of depression and mania during the natural course of affective illness, and manipulations of sleep could be used to treat and to prevent affective episodes. This project was designed to investigate the importance of the timing of sleep in patients' clinical responses to sleep manipulations. In particular, we hypothesized, on the basis of previous findings, that being awake or asleep in the second half of the night was critical for the responses. Identification of a critical circadian phase for these effects of sleep would provide an important clue to their mechanisms and would facilitate the design of effective and practical sleep deprivation treatments for depression.

The antidepressant response to partial sleep deprivation early in the night (PSD-E) was compared with the response to partial sleep deprivation late in the night (PSD-L) in 16 drug-free depressed inpatients using a balanced order crossover design. PSD-L had a significantly greater antidepressant effect than PSD-E. The response to PSD-L was sustained and enhanced by a second night of treatment. Patients had significantly shorter sleep durations and reduced REM sleep on PSD-L that did not occur on the PSD-E condition. There was a significant negative correlation between response to PSD and sleep duration, and in particular, REM sleep duration, on the late sleep deprivation condition. Thus, the amount and timing of sleep appear to be factors in the response to PSD, and the effect of this procedure on REM sleep may be important.

Project Description:

This project has been extensively described in Z01 MH 02201-04 CP. Briefly the objectives of this study were:

- 1) To determine the importance of the timing of the sleep period in the antidepressant response to PSD.
- 2) To describe the diagnostic, biochemical and neuroendocrine predictors of sleep deprivation.
- 3) To assess the therapeutic efficacy of a sustained course of treatment (3 weeks) with PSD.

Methods:

The methods have been extensively described in Z01 MH 02201-04-CP.

Significance to Biomedical Research:

- 1) The spontaneous changes in sleep that occur in depression and mania are closely associated with switches from one mood state to the other and affective disorders may alternatively be characterized as disorders of the sleep-wake regulatory system. Treatment with TSD and PSD exogenously impose a 'manic' like sleep schedule and have acute antidepressant effects in both bipolar and unipolar patients. These studies will contribute to our understanding how changes in the sleep-wake cycle result in alterations in mood perhaps through changes in neurochemistry and neuroendocrinology.
- 2) Current pharmacotherapy of depression requires approximately three weeks to work whereas the response to PSD begins immediately. If the clinical effects of PSD were sustainable, it would considerably shorten the period of morbidity and potentially decrease the risk of suicide in depressed patients.

Proposed Course:

The study has been completed, and the project is terminated.

Publications:

Sack DA, Duncan W, Rosenthal NE, Mendelson WB, Wehr, TA: The timing and duration of sleep in partial sleep deprivation therapy of depression. *Acta Psychiatrica Scandinavica*, in press.

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antidepressant Effects of Light in Seasonal Affective Disorder and Normal Controls

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	N. Rosenthal	Chief, Unit on Outpatient Studies	CPB/NIMH
Others:	R. Skwerer,	Medical Staff Fellow	CPB/NIMH
	K. Kelly,	Medical Staff Fellow	CPB/NIMH
	S. Kasper	Guest Researcher	CPB/NIMH
	P. Schulz	Social Worker	CPB/NIMH
	S. Rogers	Registered Nurse	CC/NURS
	A. Yancey	Guest Worker	CPB/NIMH

COOPERATING UNITS (if any)

Morris Waxler, Ph.D., Food and Drug Administration
 George Brainard, Ph.D., Jefferson Medical College

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

1.5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

We have previously shown that exposure of the eyes to bright light, but not ordinary room light, can reverse the winter depressive symptoms in patients with seasonal affective disorder (SAD). This light treatment may be effective during the morning, the evening or even during ordinary daylight hours. Normal subjects with no history of winter difficulties do not seem to benefit from this light treatment. This past year we have gone on to study whether light in a narrow band of the visual spectrum (blue or red) is as effective as the full-spectrum light used previously. We have also asked whether normal subjects who complain of mild winter difficulties might benefit from conventional bright light treatment.

In a crossover study on 21 subjects we found that although full-spectrum light tended to be more effective than either blue or red light, this did not reach statistical significance, perhaps because the narrow-band light was clinically active to some degree. If this is so, it would suggest that the antidepressant effects of light in SAD are mediated by wavelengths that span the full visual spectrum. A separate study of full-spectrum light in 40 normal subjects, 20 with and 20 without a history of mild winter difficulties, such as low energy level or decreased productivity, showed that the former group benefited from light treatment whereas the latter group did not. The degree of improvement was related to the duration of treatment, 5 hours per day proving superior to 2 hours per day. Since there seems to be a high prevalence of normal subjects with mild winter difficulties, this finding may have widespread public health implications.

Project Description and Methods:

1. The colored light study:

We have previously found that patients with SAD show antidepressant responses to bright full-spectrum light exposure mediated via the eye. In this study we attempted to evaluate which part of the spectrum was important in mediating this antidepressant response. Twenty-one patients with SAD were followed in our outpatient clinic until they became depressed, at which time they entered a balanced-incomplete-block treatment study, in which each patient was given two out of three possible treatments for one week each with at least one week of withdrawal between treatments. The three treatments consisted of full-spectrum, red and blue light. All sources used were fluorescent and the intensity as measured by number of photons per second per unit of surface area was kept constant across the three conditions by modifying the number of fluorescent tubes used and the distance away from the light source at which the subject was asked to sit. Light treatments were administered for two hours each morning and two hours each evening. Subjects were asked not to alter the times of sleeping and waking during the treatment week. Mood was monitored by Hamilton Depression Rating Scale (HDRS) ratings, administered by blind raters. In order to evaluate patients' expectations of treatment, we administered appropriate questionnaires before each treatment condition was started.

2. The effects of light on mood in normal subjects

This study was planned as a follow-up on the study of the previous year, in which neither bright nor dim light, administered for two hours in the early morning, was shown to have any mood-altering effects in 22 normal subjects. In that study subjects with any history of affective vulnerability or difficulties in relation to the changing seasons were specifically excluded. However, it had come to our attention that many individuals generally regarded as normal, may nonetheless experience problems in relation to the changing seasons. We also wondered whether the amount of light used in the earlier study, namely two hours per day, was insufficient to produce mood changes in normal subjects.

In this study we recruited forty subjects, twenty of whom were regarded as completely normal, having no personal or family history (first-degree relatives) of any psychiatric disturbance, nor any complaint of regular difficulties in mood or behavior during the winter months. The other twenty, which we designated as subsyndromal SAD (S-SAD) subjects, met the following inclusion criteria: (1) They had never been treated for depression during the winter months, had never regarded themselves as suffering from a disorder, nor had anyone else suggested that they should seek treatment; (2) They had a history of winter difficulties, such as impaired quality of life or decreased ability to function, for at least four weeks during at least two consecutive winters. Both types of normal subjects were recruited by appropriate, separate advertisements in the media. Before receiving light treatment all subjects were given a standardized, self-administered questionnaire, inquiring into their history of seasonal changes in mood and behavior. This questionnaire, the Seasonal Pattern Assessment Questionnaire (SPAQ) was developed in the Intramural Research Program of the National Institute of Mental Health.

Each group of subjects was further subdivided into two groups, one of which received a total of two hours of light per day (one hour in the morning and one hour in the evening), and the other of which received five hours of light per day (2.5 hours twice a day). The study was conducted as a parallel design during the winter months, each group receiving only one treatment regimen. Subjects were asked not to alter the times of sleeping and waking during

as well as by self-ratings (Profile of Mood States and Visual Analog-type ratings). Subjects' a priority predictions were again assayed.

Findings to date:

1. The colored light study:

Fourteen subjects in each study condition, a total of 21 subjects, were studied. There was a trend towards a superior response to the full-spectrum condition. However, this did not reach statistical significance. Although some response was noted following both red and blue light conditions, it was not possible to say whether this represented some biological effect of these wavelengths of light or whether the results were simply due to a placebo effect. It is possible that the lack of statistical significance was a function of the small sample size and that the negative result was a Type II error. Further studies of this kind would clearly require large numbers of subjects. In addition, green-yellow light, the color perceived as brightest by the retina or green light, the color to which the rods are most sensitive, would be interesting controls for the red and blue light in future studies.

There was no positive correlation between the subjects' a priori expectations, as determined by standardized questionnaires, and outcome. In fact, there was a negative correlation between expectations of both red and blue light and outcome following these treatments. This finding adds to the mounting evidence that prior expectations do not adequately explain the antidepressant effects of light therapy.

2. The effects of light on mood in normal subjects

We found that neither 2 nor 5 hours per day of light treatment produced a significant change in the normal group, who had reported a low level of changes in mood and behavior on history. This corroborated our finding of the year before that light does not act as a euphoriant in asymptomatic normals, like cocaine or amphetamine. Rather, it appears to help only those individuals who complain of decreased energy or other behavioral changes in the winter, even if these changes fall into the range of functioning generally regarded as normal. Such functional impairment can be determined retrospectively by means of a simple self-administered questionnaire, the Seasonal Pattern Assessment Questionnaire (SPAQ), which elicits retrospective information about level of functioning in different seasons. In these subsyndromal SAD individuals it appears that a total duration of five hours of light exposure, divided into two daily dosages, is more effective than two hours of treatment, similarly administered.

Significance to Biomedical Research:

Of the two studies of light therapy performed this past year, the study of light in normal subjects has more far-reaching implications. The findings of this study suggest that the scope of individuals who stand to benefit from light treatment is greater than was formerly appreciated. It appears likely that the subsyndromal form of SAD is more prevalent than the more extreme form that has been the focus of previous studies. However, the individuals who suffer from the less extreme form of the condition are less likely to seek medical help for their problems. In fact, one of our criteria for selecting this population was that the individuals had never previously specifically sought help for their winter difficulties. Thus the problem of treating these people, by definition, falls within the realm of public health workers rather than clinicians. It is important for these workers to be aware of the existence of numerous

individuals whose performance and quality of life might well be improved by enhancing their environmental light. The development of a simple self-administered retrospective questionnaire, the SPAQ, is a valuable tool in the definition of this population. It has already been widely used by fellow researchers, who have found it to be of value. Christopher Thompson, at Charing Cross Hospital in London, has found that the questionnaire differentiates between SAD patients, other affective disorder patients and normal individuals. Dr. Michael Terman, at Columbia University in New York City, has performed an epidemiological survey in which he has mailed this questionnaire to people randomly chosen from the Manhattan telephone directory. He has found that at least 25% of those who returned their questionnaires (57% of the surveyed population responded) complain of seasonal mood and behavior problems of severity equal to or greater than the S-SAD individuals described above. Based on our studies of the effects of bright light in normals, Dr. Terman has projected that approximately 1.9 million people in the New York Metropolitan Area would benefit from enhancement of their environmental light. The SPAQ is currently being used in several other studies in the United States. It has also been translated into French, German and Russian, and is being used in ongoing studies in Europe and the United States.

While our recent study of normal individuals showed that some normals benefit from enhanced light exposure, it also confirmed our earlier finding that normal individuals without a history of seasonal changes in mood or behavior do not appear to benefit from enhanced environmental lighting. This finding would suggest that indiscriminate enhancement of environmental lighting in the work and living environments of all people is not warranted. Rather, some way of selecting those individuals who would benefit from enhanced environmental lighting is desirable, and the SPAQ would seem to be a logical instrument to use in such a selection process. Enhanced environmental lighting should be recommended for those with high scores on this questionnaire.

The results of the colored light study were equivocal. Although there was a suggestion that the full-spectrum light was superior to both red and blue lights, this did not reach statistical significance perhaps, in part, because the colored lights had some real biological activity of their own. The partial efficacy of blue and red light might imply that the antidepressant effects of light in SAD are induced by a wide array of wavelengths within the visual spectrum. However, it remains to be determined which wavelengths exert the most powerful effect in this regard.

Proposed Course:

Further studies of the antidepressant effects of light can be conceptualized along two broad lines: 1. the ongoing definition of which individuals are most likely to benefit from light; and 2. continued investigations of the formal properties of light involved in phototherapy. These broad areas will be addressed in turn.

1. Who stands to benefit from enhanced environmental light?

A. The General Population: As discussed above, we have more closely defined the range of individuals who may benefit from enhanced environmental lighting. We plan to extend the approach taken by Terman in his Manhattan project with two modifications: (1) We plan to use state-of-the-art epidemiological techniques for studying the prevalence of seasonal changes in mood and behavior in the population of Montgomery County. This would involve the use of random-digit dialling and telephone interviewing, using the SPAQ, of a randomly selected sample by an outside group of experts in these techniques. Once the sample has been defined,

we intend to approach randomly selected individuals within subsamples of the larger population, based on the severity of their seasonality as determined by SPAQ scores, and offer them light treatment in the winter months. In this way we hope to project accurately what proportion of the population, who do not seek out any medical or psychiatric intervention, would benefit from having their environmental light enhanced.

In addition to the above study, less ambitious surveys involving questionnaires sent by mail and administered to patients waiting to be seen in doctors' offices, are in progress, in collaboration with the Psychiatric Institutes of America, to evaluate the prevalence of seasonal problems at three different locations at different latitudes: Nashua, New Hampshire; Washington, D.C.; and Sarasota, Florida.

B. The elderly: We are planning to study the effects of bright light in an elderly population, not selected on the basis of prescreened seasonality. It has come to our attention that the symptoms of SAD frequently become worse over time. It is unclear what factors are involved in this symptomatic deterioration but declining eyesight and decreased perception of brightness may be relevant. The migration of the elderly to Florida and other milder climates, especially in the winter months, is a well-recognized phenomenon. This migration has popularly been attributed to difficulty with the cold rather than to deprivation of environmental light. However, it is quite conceivable that the latter factor plays an important role in the discomfort that prompts migration to the south. We recognize that not all elderly individuals are fortunate enough to be able to move south. The institutionalized elderly come to mind immediately in this regard. Unable to get outdoors easily during the winter, they are particularly vulnerable to the behavioral and mood effects of light deprivation. We plan to study the effects of light on a selected group of elderly individuals and to explore the degree to which this may improve the quality of their lives. It would be important in such a study, as in all efficacy studies, to control for the influence of non-specific placebo factors in the behavioral response to lighting interventions.

2. Studies of the Formal Properties of Light Required for Effective Phototherapy

Studies previously undertaken in the Intramural Research Program have examined several aspects of the formal properties of effective phototherapy. For example, we have found that, in order for light to be effective, it should be of high intensity and that the eyes rather than the skin should be exposed to the light. We have also shown, in a series of studies, that the timing of light treatment is not crucial for its effects. Most recently we explored the spectrum of light required for effective phototherapy. At this time we have not decided whether we shall undertake further studies of the formal properties of light required for effective phototherapy in the coming year.

An important unresolved issue is whether the small amount of ultraviolet light present in the full-spectrum light used in most studies performed to date, is necessary for its antidepressant effects. Preliminary findings both from Dr. Alfred Lewy in Oregon and from Dr. John Docherty in New Hampshire suggest that this is not the case. Our group has been involved as collaborators in the latter of the two studies. We are planning to continue the New England collaboration in the forthcoming year, and Dr. John Docherty has recruited the support of several mental health centers in comparing light sources with and without ultraviolet light. The importance of this question grows as it becomes clear that some individuals require enhanced environmental lighting for several hours a day for the larger part of the year, perhaps for most of their lives. We know that in excessive dosage ultraviolet light has potentially harmful effects on both the eye and the skin. If it can be definitively established that ultraviolet light is

unnecessary for the mood-altering and behavioral effects of enhanced environmental lighting, UV light should be excluded from the light sources in future. At present, however, there is inadequate data on which to base such widespread recommendations.

There are many outstanding questions pertaining to the formal properties of light treatment. What is the optimal duration, intensity, timing, and spectrum of light? What is the safest, cheapest and most convenient way to administer light therapy? All of these questions are eminently testable and would almost certainly lead to useful practical information. The major question in setting our research priorities is whether, given finite resources, we will be able to pursue these research areas as well as investigate the biological effects of light therapy administered in a standard fashion. This latter approach has yielded exciting preliminary findings (see Annual Report # Z01 MH 02206-03 CP) and we plan to pursue it further. Now that the efficacy of phototherapy has been widely confirmed by a number of groups both in the U.S. and Europe, several groups are in the process of undertaking the types of formal studies mentioned above. We have not as yet decided whether we in the Intramural Program of the NIMH should continue in this direction or whether, at this time, it is better to leave these clinical trials to others while we pursue the more specialized psychobiology studies we are equipped to do.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02206-03 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Seasonal Affective Disorder and Light Therapy

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although it has been established that bright light is an effective treatment for SAD, the mechanism of its action remains unknown. We have shown that our previous theory, that light works by suppressing melatonin secretion, is an unsatisfactory explanation of this form of treatment. Another theory, proposed by Lewy and colleagues, that light exerts its antidepressant effects by modifying circadian rhythms, is similarly unsatisfactory. We have continued to pursue our investigations by measuring relevant biological variables in SAD patients and normal subjects and evaluating the effects of light on these measures.

We have found several differences in patients with SAD and normals: (1) Increased mitogen stimulation of peripheral lymphocytes in both summer and winter in patients compared to normals; bright light suppresses this response in patients but enhances it in normals. (2) SAD patients show increased resting metabolic rate (RMR) in winter compared to normals, and light significantly suppresses the RMR. (3) Plasma norepinephrine levels in SAD patients are inversely proportional to the severity of their depressive symptoms, and increased norepinephrine levels are observed in response to light treatment in direct proportion to its antidepressant efficacy. (3) A similar direct relationship between treatment efficacy and a biological effect is observed in the amplitude enhancement of the P300 component of the event related potential in SAD patients, a change seen within 48 hours of beginning light therapy. (4) Brain serotonin pathways appear to be abnormally sensitive in SAD patients as measured by psychological and hormonal responses (prolactin and cortisol) to injection of the serotonin agonist, M-CPP. (5) Overnight plasma melatonin is low in SAD, as in other depressed populations. (5) Light treatment appears to delay the nadir of cortisol secretion. (6) Recent sleep studies show a shorter total sleep time and greater REM percentage and movement time in patients compared to controls. Light increases delta sleep time and sleep efficiency in patients but decreases these parameters in control subjects. These biological findings are discussed.

Project Description and Methods:

1. Study of the Psychobiological Effects of Light

This study was continued from the previous winter. Since light is capable of inducing rapid and marked antidepressant effects in SAD patients, we evaluated the effects of light on systems we regard as relevant to the psychophysiology of SAD, by comparing "on-light" and "off-light" conditions in patients. Similar measurements in normal controls permitted us to evaluate patient-normal differences.

SAD patients were followed longitudinally from the summer and fall, when they were euthymic, into the winter. As they became depressed, they were assigned to one of two conditions on a random basis: off-lights first or on-lights first, to avoid an ordering effect. This assignment determined whether psychobiological tests were performed before light treatment in the first instance or on light treatment at first. In order to be included in the study, patients were required to have been off lights or on lights for at least 10 days before coming into our inpatient unit. Most of the patients were drug-free but a few patients on medications were included provided they had been on the medications for an extended period of time and the dosage could be held constant throughout the study. After the initial set of studies, patients were crossed over to the alternate condition. Age- and sex-matched controls were recruited and studied in the same way as the patients.

Studies consisted of 24-hour profiles of a variety of plasma hormones, drawn via an indwelling intravenous catheter, EEG-recorded sleep studies for 2 nights (excluding an adaptation night), mitogen stimulation testing of lymphocytes in vitro, plasma norepinephrine measurements in both recumbent and standing positions, event related brain potentials and reaction time, and a lumbar puncture. An additional study performed this year but not last was the resting metabolic rate (RMR), measured by indirect calorimetry. Not all subjects agreed to go through all studies. The lumbar puncture was performed on the last day in the morning, after a night of bedrest and with patients in a fasting state. Thirty-two mls of CSF were removed and these were assayed for the monoamine metabolites, MHPG, 5-HIAA and HVA, and the peptide neuromodulators, neuropeptide Y, peptide YY and growth hormone releasing hormone (GHRH). Blood specimens were analyzed for prolactin, growth hormone, cortisol, melatonin and thyroid stimulating hormone (TSH). The immunological studies that were performed as part of this study are reported elsewhere (See Annual Report #Z01 MH 0235-02 CP).

In order to obtain event related brain potentials (ERPs), subjects were tested with auditory and visual versions of three reaction time tasks; 0.10/0.90 stimuli were used to elicit P300s (the component of the ERP that occurs 300 msec after the presentation of the stimulus). Control subjects were also tested twice. Eleven channels of EEG as well as EOG were recorded and digitized at the rate of 200 Hz over an 1100-msec recording interval (See Annual Report # Z01 MH 00509-05 LPP). A subset of 6 patients and 2 normals were tested several times over the course of light therapy, on days 2, 3 and 10 of treatment, in order to define the time course of the enhancement of the P300 component in relation to the change in mood.

2. Neuroendocrine Challenge with m-Chlorophenylpiperazine (mCPP)

There are many clues that point to the possible pathophysiological importance of serotonergic abnormalities in SAD. Such abnormalities have long been postulated to be an important etiological factor in depression in general. Swedish researchers have found that hypothalamic serotonin content in post-mortem human brains drops to its lowest in the winter months.

Carbohydrate intake has been linked to brain serotonin metabolism in both animals and humans, and we have suggested that the carbohydrate craving seen in SAD may represent a behavioral attempt to enhance deficient serotonergic functioning. These lines of reasoning have led us to challenge SAD patients and normals with serotonin agonists.

In a previous study we evaluated the effects of 5-Hydroxytryptophan (5-HTP) in SAD patients and normals but found no difference in their neuroendocrine responses. Both groups showed no change in plasma cortisol but a relative suppression of prolactin secretion following 5-HTP. In order to discriminate more effectively between neuroendocrine responses in patients and normals, we recently administered the more selective serotonin receptor agonist, m-chlorophenylpiperazine (mCPP) before and after light treatment i.e., in depressed and remitted states.

10 depressed patients with SAD and 11 controls matched by age, sex, weight, and menstrual history (ie pre- or post-menopausal) were given an intravenous bolus of mCPP (0.1mg/kg) before and after treatment for a week with phototherapy. All subjects were drug-free for more than 3 weeks, phototherapy-free for more than 2 weeks, and maintained a strictly controlled sleep schedule (6:00-6:30 a.m. wake-up) and environmental light exposure beginning at least one week prior to the first infusion day. On each infusion day two IVs were started at 8:30 a.m. and blood samples were drawn at -10, 0, 2, 5, 10, 20, 30, 40, 50, 60, and 90 minutes in relation to an infusion of mCPP at 10:00 a.m. Blood pressure and pulse were recorded every 5 minutes and oral temperature was recorded at -15, 0, 30, 45, 60, 90 minutes. Subjects completed the following self-rating scales at -15, 30, 60, and 90 minutes: NIMH 24-item scale (6 composite items: activation-euphoria, depressed affect, anxiety, dysphoria, altered self, functional deficit), Drug Effects Rating Scale, Stanford Sleepiness Scale, and 15 Visual Analog Scale (VAS) 100 mm line items. Serum samples were assayed for prolactin and cortisol by RIA, and for mCPP blood level by HPLC. Prolactin, cortisol, temperature recordings, and self-rating scale scores were analysed by ANOVA, and baseline and Δ_{max} values were compared by t-tests. During the week between the two mCPP infusions, phototherapy (2500 lux, 4 hours per day) was given at home or work.

3. Photo-Immune Studies in SAD and Normal Subjects

Mitogen stimulation was performed on lymphocytes by treating them with the mitogens, Concanavalin A, phytohemagglutinin, or pokeweed mitogen (see Annual Report Z01 MH 02325-01 CP for further details).

Findings to Date:

1. Study of the Psychobiological Effects of Light

Overnight studies of plasma hormones showed significantly reduced peak melatonin levels ($P < .05$) and a non-significant tendency to delayed onset of melatonin secretion in patients with SAD compared to normal controls. Light treatment delayed the nadir of cortisol secretion by approximately one hour ($P < .05$).

Sleep Studies were performed in 10 SAD patients and 6 normal controls both on and off light treatment. Group differences were present in total sleep time, which was less in patients than in normals; and REM time, REM percent and movement time, which were greater in patients than in normals. Significant interactions between subject group and light condition were found for sleep

efficiency ($P=.05$) and delta sleep and delta percent ($P=.02$). Light increased delta sleep and sleep efficiency in SAD patients but decreased these parameters in normal controls. Plasma norepinephrine levels in SAD patients were inversely related to their level of depression off light treatment, as measured by the HDRS ($N= 14$; $r = - 0.75$; $P<.05$). The increase in resting plasma norepinephrine during the 'on-light' condition was directly proportional to the improvement in depression level, as measured by the HDRS ($N= 13$; $r = 0.59$; $P<.05$).

Resting Metabolic Rate (RMR) in 10 SAD patients tended to be higher than in 9 normal controls during the off-light condition (1648 versus 1357 Kcal/day; $P<.10$). Light treatment significantly decreased RMR in patients by an average of 201 Kcal/day ($P<.05$) but had no significant effect on RMR levels in normals.

The enhancement of the P300 component of the Event Related Brain Potentials (ERPs) in response to visual stimuli, observed the previous year in a small number of patients ($N=7$) was sustained in a larger number ($N=17$). This finding was observed in tracings from three different scalp sites and while subjects were performing two different tasks. Correlations were between 0.5 and 0.6, with significance values ranging between $P<.05$ and $P<.001$. Once again, we observed no such correlation in the P300 change and mood in auditory ERPs. We studied the time course of changes in the ERPs in response to light treatment in 6 SAD patients and 2 normal controls, who were tested several times during light therapy as well as at baseline. The enhancement of P300 was found to occur in SAD patients as early as 2 days after light therapy was started, and increased with increasing antidepressant response to treatment.

Lumbar punctures were performed on a total of 11 SAD patients on and off light treatment and 9 normal controls off light treatment. No differences between groups or conditions were observed in the amine metabolites, MHPG, HVA and 5-HIAA, or in the peptides, neuropeptide Y, peptide YY or Growth Hormone Releasing Hormone.

2. Administration of M-CPP before and after light treatment

Effects of Light Treatment on Mood: Patients' Hamilton Depression Rating Scale (HDRS) scores (mean \pm S.D.) fell significantly over the course of phototherapy, from 17.3 ± 3.7 on the first infusion day (TD1) to 7.9 ± 4.3 on the second infusion day (TD2) ($t=6.33, P<0.001$).

NIMH 24-item self-rating scale: ANOVAs of the mean composite NIMH-24 scale scores of patients and controls revealed a significant *group x day x time* interaction for "activation-euphoria" ($p<0.01$). Patients showed a significantly greater rise in "activation-euphoria" following mCPP than controls, particularly on TD1 (prior to phototherapy). Patients' mCPP-stimulated "activation-euphoria" normalized on TD2 (following phototherapy and relief of depressive symptoms).

Patients' ratings of depressed affect were significantly higher than controls' ratings ($p<0.001$) on both TD1 and TD2, and ANOVA failed to reveal significant interactions. "Depressed affect" trended to be higher in both groups on TD1 than TD2 ($p<0.1$) and also varied significantly over time for both groups ($p<0.05$). Both patients and controls showed significantly higher mCPP-stimulated values on TD1 than on TD2 on the following factors: altered self, anxiety, and functional deficit. The mean time course of ratings (group x time interaction) also differed significantly between the groups for "altered self" ($p=0.0001$), "anxiety" ($p<0.005$), and "dysphoria" ($p<0.005$).

Drug effects rating scale: Patients' mean drug effects ratings differed significantly from those of controls over time on the two infusion days (group x day x time interaction) for "slowed down" ($p=0.0001$) and "drowsy" ($p=0.051$), with patients starting from higher baselines and having less mCPP-stimulated slowing and drowsiness than controls on TD1. On TD2, the patients' mCPP-stimulated ratings of "slowed down" and "drowsy" were similar to those of controls.

Patients and controls also reported significantly more of the following side-effects over time on TD1 than TD2 (day x time interaction): chilled ($p=0.0001$), light-headed ($p<0.005$), trouble remembering ($p<0.05$), yawning ($p<0.05$), and hot/flushed (trend, $p=0.06$).

Stanford Sleepiness Scale: Patients were sleepier than controls on both days, but showed a trend for less mCPP-stimulated sleepiness than controls on TD1 (group x day x time interaction $p<0.06$).

Temperature: no significant baseline differences were observed between patients and controls and ANOVA revealed no significant interactions or group differences. There was a trend ($p<0.1$) for patients' Δ_{max} TD2 temperature to be greater than that of controls.

Hormone Studies

A. Cortisol: Patients showed a trend ($p<0.1$) towards higher basal serum cortisol levels than controls on TD1 and showed significantly higher basal cortisol levels than controls on TD2 ($p<0.05$). Both patients and controls showed robust stimulation of serum cortisol in response to IV mCPP. The mCPP-stimulated cortisol rise was significantly greater over time in patients than in controls on both test days ($p<0.0005$). mCPP-stimulated cortisol levels were also significantly higher on TD1 than on TD2 in both groups ($p<0.005$). Patients' Δ_{max} [peak-baseline] cortisol levels showed a trend to be higher than controls' levels following the second ($p<0.06$) but not the first infusion. Patients, but not controls, showed a significant decrease ($p<0.01$) in Δ_{max} cortisol levels from TD1 to TD2.

B. Prolactin: There were no differences in basal serum prolactin levels between SAD patients and controls on either test day. Both patients and controls showed robust increases in serum prolactin in response to mCPP. The mCPP-stimulated prolactin rise was significantly greater over time in SAD patients than in controls on both test days ($p<0.005$). mCPP-stimulated prolactin levels were significantly higher on TD1 than on TD2 in both groups ($p<0.005$). Controls, but not patients, showed a significant decrease in Δ_{max} prolactin levels from TD1 to TD2 ($P<0.05$). Patients' Δ_{max} prolactin levels were higher than controls' on TD2 ($p<0.05$) but not on TD1.

Thus depressed SAD patients showed higher basal and mCPP-stimulated cortisol levels, and also higher mCPP-stimulated prolactin levels than matched controls. The higher cortisol and prolactin levels of patients were present in both depressed and remitted states, suggesting the possibility that heightened sensitivity to changes in serotonergic function may be a trait marker in SAD patients. We should note, however, in arguing against this idea, that patients did not remit completely following light therapy. The current findings differ from those of our earlier study in which depressed SAD patients showed higher basal but equivalent 5-HTP-stimulated cortisol and prolactin levels than controls. The difference in the stimulated hormonal levels in the two studies may reflect the greater serotonergic selectivity of mCPP.

As with the hormonal findings, SAD patients' subjective responses to mCPP were greater than

controls' responses on many (but not all) items. The items "activation-euphoria", "slowed down", and "drowsy" were of particular interest since patients' mCPP-stimulated ratings tended to normalize with phototherapy and improvement in depression. These changes in subjective response to mCPP may signify functional serotonergic differences between depressed SAD patients and controls which are normalized by exposure to phototherapy. The finding that mCPP produces less slowing and drowsiness in SAD patients than in normal controls is reminiscent of our earlier finding that carbohydrate-rich meals also show a similar differential subjective effect on these two groups. Both findings suggest a difference in the serotonin system of SAD patients and normals.

Both patients and controls showed decreased hormonal and [in many cases] decreased subjective responses to mCPP on the second infusion day compared with the first infusion day. A second group of controls (N=6) given IV mCPP on two mornings a week apart without exposure to phototherapy showed similar decreases in both hormonal and subjective responses. Our preliminary data analysis levels suggests that these effects may represent a down-regulation of serotonergic receptors present a week after a single exposure to IV mCPP.

The abnormal serotonergic function in SAD patients and its normalization by light, suggested by the above study, is reinforced by recent findings from other groups, who have shown the serotonin agonist, D-fenfluramine, and the serotonin precursor, L-tryptophan to be somewhat effective in the treatment of SAD. Taken together, all these findings suggest that abnormalities in serotonin metabolism may be an important etiological factor in SAD and that light therapy may work, at least in part, by correcting this abnormality.

3. Photo-Immune Studies in SAD and Normal Subjects

Peripheral blood lymphocytes (PBL) were stimulated to a greater degree in SAD patients than in normals during both the winter and the summer. Seven to ten days of light treatment was associated with a decrease of PBL stimulation to normal levels. In 10 normal subjects bright (2500 lux) but not dimmer (300 lux) light was associated with significant increase in PBL stimulation and since the skin was completely covered in this study, this effect was evidently mediated via the eyes. For further details see Annual Report #Z01 MH 0235-02 CP.

Significance to Biomedical Research:

The definition of the syndrome of SAD and the recognition of its response to light have been followed by new insights into the neurobiology of this subset of affective disorders and rapidly accumulating information on the biological effects of light in humans. Light has emerged as a valuable probe into the understanding of brain functioning.

Patients with SAD differ from commonly reported affective disorder patients in that they typically overeat, oversleep and gain weight. It appears as though their biological features may also differ in some ways. For example, typical affective disorder patients have been shown to have decreased peripheral blood lymphocyte (PBL) stimulation, whereas in SAD patients this function appears to be increased. The inverse relationship between plasma norepinephrine levels and severity of depression, reported here in SAD patients, has not been consistently reported in more typical affective disorder patients. However, other biological findings reported here, namely low nocturnal melatonin secretion and decreased total sleep time, have been reported in classical depressives. We should note that we have previously reported total sleep time to be increased during the winter in SAD, a confirmation of patients' clinical

sleep time to be increased during the winter in SAD, a confirmation of patients' clinical observation. It is only a minority who report decreased sleep time and it is conceivable that this minority was overrepresented in the present sample of patients studied.

The many observed biological effects of light continue to add to our knowledge of this environmental variable, only recently regarded as inert as far as human brain functioning was concerned. The earlier observation that light enhances the P300 component of the visual ERP has been sustained in a larger sample size, as have the suppressing effects of light on PBL function in patients with SAD. It appears that the antidepressant effects of light correlate with improved processing of visual, but not auditory, information. This objective correlate of the antidepressant response to light appears to hold promise for future studies, especially since it can be observed as early as 48 hours after starting light treatment.

An unexpected effect of bright light was the reduction of RMR in patients. Many of the symptoms of SAD, such as increased sleeping, decreased activity, overeating and weight gain, suggests energy-conserving strategies. Since light treatment reverses these symptoms, thus decreasing energy conservation, we had predicted that it would increase RMR. The decrease in RMR is difficult to explain but it may be secondary to the decreased appetite and weight loss that occur in response to treatment. We should note that antidepressant medications have been shown to decrease RMR in non-seasonal depressives.

The significant reduction in RMR seen following light treatment in SAD patients was not observed in normal subjects. Different effects of light in patients and normals were also seen on PBL function, on sleep, and on stimulation of the serotonin system by M-CPP. Bright light suppressed PBL function in SAD patients in the winter but increased it in normal subjects. Similarly bright light increased sleep efficiency and delta sleep in patients but decreased these parameters in normals. Such differential effects of light on SAD patients and normals may provide a clue to the pathophysiology of SAD. The exaggerated sensitivity of the serotonin system to stimulation with the post-synaptic serotonin agonist, M-CPP, provides one more piece of evidence that serotonergic function may be abnormal in SAD and that alterations in this system may partially explain the antidepressant effects of light.

The phase-response theory as an explanation for how light therapy works, is not substantiated by our data. Although there was a non-significant tendency for SAD patients to show a delayed onset of melatonin secretion when compared to controls, there was no evidence from hormonal or sleep data, that light treatment advances circadian rhythms, the effect that has been hypothesized to underlie its antidepressant properties. On the contrary, effective light treatment was seen to delay the cortisol nadir in SAD patients.

Initially in the study of SAD and phototherapy, there appeared to be few biological markers of the condition. The traditional biological abnormalities found in depression did not appear to be present in SAD. Plasma cortisol levels, the response to the dexamethasone suppression test, and REM latency were normal. We have now shown several biochemical and physiological abnormalities in SAD patients, both at baseline and in response to light. A problem for future research efforts will be to understand better the nature of these abnormalities, to sort out which will reveal most about the fundamental disturbance of this condition and, most important, to attempt to weave a coherent story out of these many abnormal findings.

Proposed Course:

The comparison of SAD patients and normals on and off light treatment has been an extremely fruitful approach and several physiological systems appear to differentiate subjects and conditions. Further studies are suggested to explore these earlier findings in greater depth. How do patients and normals differ across the seasons? What are the effects of light on norepinephrine levels in normals? Will challenges to the RMR elicit further patient-normal and on-light /off-light differences, for example a study of diet-induced thermogenesis? What is the precise nature of the lymphocyte changes induced by light? Are there changes in phenotype of B- or T-Cells, or are all lymphocytes affected similarly? Do the observed in vitro changes in immunity correspond to any in vivo changes? Can the P300 enhancement provide information about an immediate physiological effect of light? If so, does this effect show a circadian variation, and is it a predictor of long-term antidepressant effects of light? How can we best follow up the abnormalities in the norepinephrine and serotonin systems and their apparent normalization by bright light treatment? Many questions present themselves; the challenge will be to choose those questions most likely to reveal the fundamental abnormality in SAD and best elucidate the biological effects of light.

Publications:

Wehr TA, Sack DA, Jacobsen F, Tamarkin L, Arendt J, Rosenthal NE. Timing of phototherapy and its effect on melatonin secretion are not critical for its antidepressant effect in seasonal affective disorder. *Archives of General Psychiatry* 43:870-875, 1986.

James SP, Wehr TA, Sack DA, Parry BL, Rogers SL, Rosenthal NE. The dexamethasone suppression test in seasonal affective disorder. *Comprehensive Psychiatry* 27:224-226, 1986.

Rosenthal NE, Sack DA, Jacobsen FM, James SP, Parry BL, Arendt J, Tamarkin L, Wehr TA. The role of melatonin in seasonal affective disorder. *Journal of Neural Transmission* (suppl) 21: 257-267, 1986.

Sack DA, Rosenthal NE. Do changes in melatonin cause SAD? Commentary submitted to *Integrative Psychiatry*, Oct. 1986.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02222-01 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Treatment of Rapid-Cycling Manic-Depressive with Thyroxine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Sack	Chief, Inpatient Services	CPB/NIMH
Others:	T. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH
	N. Rosenthal	Chief, Outpatient Services	CPB/NIMH
	W. Mendelson	Chief, Unit on Sleep Studies	CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3

PROFESSIONAL:

2

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There is some evidence that the hypothalamic-pituitary-thyroid axis plays a role in the pathophysiology of affective illness, particularly in patients with rapid cycling forms of manic-depressive illness, who have a very high incidence of lithium-induced hypothyroidism. Rapid cycling patients are often refractory to standard types of mood-stabilizing treatments. Since the 1930s there have been three reports that hypermetabolic doses of thyroxine suppress rapid cycling. These reports were based on uncontrolled studies.

We treated seven rapid cycling patients with suppressive and hypermetabolic doses of thyroxine in a placebo controlled, double-blind study. No patient improved on suppressive doses. Two patients remitted completely on hypermetabolic doses, but both eventually relapsed after many months of treatment. This result is interesting from a theoretical point of view, but does not seem to offer any new approach to treatment for refractory rapid cycling patients. Therefore, the project has been terminated.

Project Description:

This project is designed to assess the hypothalamic-pituitary-thyroid axis (HPT) in rapid-cycling manic-depressives and to test the therapeutic efficacy of exogenously administered thyroxine in these patients. A complete description of the project and methods appears in Z01 MH 02222-03 CP.

Methods:

We have now evaluated the mood stabilizing effects of thyroxine in 7 patients in a double-blind controlled trial. No patients improved on replacement doses of thyroxine. On hypermetabolic doses of thyroxine 2 patients showed complete remission in their mood cycles. Both of these patients relapsed when thyroxine was decreased to suppressive doses and remitted when thyroxine was reinstated at the higher dose. Long term follow-up reveals that both patients eventually relapsed into rapid-cycles despite continuing on hypermetabolic treatment and showing elevated thyroid values.

Significance to Biomedical Research:

- 1) Although rapid-cyclers differ from other groups of manic-depressive patients with respect to the frequency of their episodes, they are the same with respect to clinical presentation, age of onset and genetic predisposition. The overt thyroid disease that these patients manifest may be a particularly useful model for understanding the more subtle HPT disturbances seen in other affective patients.
- 2) While rapid-cyclers constitute approximately 15% of manic-depressive patients they are largely refractory to conventional therapy and constitute a formidable problem in clinical practice. Understanding the nature of the thyroid disturbance in these patients could lead new and better therapies.

Proposed Course:

The results of this study are interesting from a theoretical point of view, but they do not seem to offer any new approach to the treatment of refractory rapid cycling patients. Therefore, the project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02223-04-CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pentobarbital and Ethanol Toxicity: Relation to the Benzodiazepine Receptor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. B. Mendelson	Chief, Section on Sleep Studies	CPB/NIMH
Others:	J.V. Martin	Chemist	CPB/NIMH
	R. Wagner	Guest Worker	CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Section on Sleep Studies

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the course of measurements of respiration, spontaneously occurring cessations of breathing, analogous to human sleep apneas, were observed in rats. Subsequently, the apparatus for monitoring respiration was dedicated to Project #Z01-MH 02382-01 CP in order to characterize an animal model of sleep apnea. For this reason, and because the investigators left NIH in July, 1987, Project Z01 MH 02223-04-CP has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02225-04-CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Role of Calcium Flux in the Sleep-Inducing Effects of Flurazepam

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. B. Mendelson	Chief, Section on Sleep Studies	CPB/NIMH
Others:	J.V. Martin	Chemist	CPB/NIMH
	R. Wagner	Guest Worker	CPB/NIMH

COOPERATING UNITS (if any)

S. Paul, Chief, Clinical Neuroscience Branch, NIMH
 M. Majewska, Visiting Associate, Clinical Neuroscience Branch, NIMH

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Section on Sleep Studies

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our previous studies have shown that there may be a functional relationship between benzodiazepine (BZ) receptor stimulation and alterations in calcium flux. Nifedipine, a calcium channel blocker, prevents the sleep-inducing effects of flurazepam in rats. Conversely, BAY K 8644, a calcium channel agonist, potentiates the effects of flurazepam on sleep. However, as we reported last year, experimental models for the anticonvulsant and anxiolytic effects of BZ's are not affected by calcium channel antagonists, indicating that calcium channels may have a more important role in sleep induction than in other actions of BZ's. The sleep-inducing effects of pentobarbital were not sensitive to inhibition by nifedipine, indicating that barbiturates may cause sleep through a mechanism that does not require changes in calcium flux. Since pentobarbital causes changes in chloride ion flux, a second mechanism for sleep induction might involve a chloride channel. To expand this line of investigation, this year we studied the effects on sleep of two steroids, which enhance chloride uptake into synaptoneurosome in a manner similar to barbiturates.

The two steroids, 3alpha, 5alpha -tetrahydrocorticosterone (THDOC) and 3alpha -hydroxy-5alpha-dihydroprogesterone (OH-DHP), are endogenously occurring ring A metabolites of corticosterone and progesterone. Since THDOC and OH-DHP not only alter chloride flux but also enhance the binding of [³H]flunitrazepam to brain membranes, we studied the effects on sleep in rats of 5 and 10 mg/kg of the steroids alone and in combination with a low dose of flurazepam. THDOC, but not OH-DHP, had potent dose-dependent sleep-inducing properties and increased nonREM sleep, but did not affect REM sleep. Flurazepam had a similar hypnotic effect but additionally reduced REM sleep. There were no significant interactions between THDOC and flurazepam.

This project was terminated in July, 1987 when the investigators in the Section on Sleep Studies left NIH.

Project Description:

Please refer to Z01 MH 02225-01-CP

Methods:

Male rats were injected intraperitoneally (IP) with 5 or 10 mg/kg OH-DHP or THDOC or with vehicle. Five minutes later the rats were given a second IP injection, of 20 mg/kg flurazepam or vehicle. Standard two-hour EEG recordings were then performed.

Findings to Date:

In the present study, OH-DHP (5 and 10 mg/kg) had no significant effect on sleep, nor did it alter the effects of flurazepam.

While the low dose of THDOC (5 mg/kg) had no significant effect on sleep, it tended to decrease sleep latency. The high dose of THDOC (10 mg/kg) significantly reduced sleep latency from 14.6 ± 2.4 min to 8.2 ± 1.9 min (mean \pm S.E.M; $p < 0.01$; $n=15$). As expected, flurazepam also significantly decreased sleep latency, to 9.4 ± 2.0 min ($p < 0.05$). There was, however, no significant interaction between THDOC and flurazepam; when both drugs were given together, the sleep latency was 6.5 ± 1.2 min. Similarly, both THDOC (10 mg/kg) and flurazepam increased nonREM sleep ($p < 0.01$ each) again with no significant interaction. Flurazepam, but not THDOC reduced REM sleep ($p < 0.05$) without a significant interaction.

Significance to Biomedical Research:

Previous studies showed a parallel between effects of BZ's on sleep and a calcium ion flux related to occupation of BZ receptors. However, the hypnotic effects of pentobarbital are not sensitive to the calcium channel blocker nifedipine, implying that a second mechanism for sleep induction is independent of the calcium channel. The present findings indicate that THDOC, a steroid compound which stimulates chloride flux, is a potent sleep inducer. Both calcium and chloride ion fluxes, therefore, deserve consideration as possible effector mechanisms for the actions of different classes of hypnotic drugs.

The finding that an endogenous corticosterone derivative has strong hypnotic qualities may have important implications for investigations of the role of the adrenal gland in stress responses. Furthermore, from a purely clinical viewpoint, the discovery of an endogenous compound which induces nonREM sleep without detrimental effects on REM sleep is unusual and may have therapeutic applications.

Publication:

Mendelson, WB, Martin, JV, Perlis, M, Wagner, R, Majewska, MD and Paul, SM: Sleep induction by an adrenal steroid in the rat. Psychopharmacology, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02290-03 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Melatonin analysis of clinical samples

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. Tamarkin	Research Biologist	CPB/NIMH
Others:	G. Paciotti	Biologist	CPB/NIMH
	M. Collins	Biologist	CPB/NIMH

COOPERATING UNITS (if any)

W. Berretini and J. Nurnberger, Clinical Neurogenetics Branch, NIMH,

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Melatonin has been measured in plasma and cerebrospinal fluid from human patients and normal volunteers. The findings of the studies outlined in last year's report are currently being written for submission to peer reviewed journals. The one major ongoing study is focused on the possible heritability of the increased sensitivity to light at night of children of parents with major depression.

Project Description:

The clinical study of the regulation of melatonin secretion is done in collaboration with Dr. W. Berettini and is an acute exposure of human subjects to approximately 500 lux of light at night. We have previously observed that the nocturnal secretion of melatonin from normal subjects is not affected by exposure to this intensity of light at night. However, depressed patients either during a depressive episode or euthymic are more sensitive to light at night and show a suppression of plasma melatonin. Children of depressed parents were the focus of this investigation and were asked to participate in a clinical study similar in design to the adult study.

Methods:

Offspring of one bipolar parent (N=18) and offspring of an affectively ill couple (one bipolar, N=7) were compared to 20 aged-matched controls with no familial history of affective disorder. Blood samples were drawn from 1am to 2am in the dark and from 2am to 4am in the light. Plasma was harvested for melatonin determination.

Findings to Date:

Significantly more subjects with one or two affected parents were sensitive to 500 lux of light at night. Suppression of plasma melatonin was noted in 3/20 subjects in the 0 parent ill group, 6/18 in the 1 parent ill group, and 4/7 in the 2 parent ill group.

Significance to Biomedical Research:

These data would suggest that there is an association of increased sensitivity to light and affective disorder, and further that this characteristic occurs more frequently in offspring of affected parents. One hypothesis that needs to be explored is that this increased sensitivity to light at night may also be predictive to increased vulnerability.

Proposed course:

Continued investigations of offspring are planned and further study of the specific mechanism for the increased sensitivity to light are being discussed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02292-03 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Melatonin Effect on Hormone-Stimulated Cell Growth

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Tamarkin Research Biologist CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Project held in abeyance

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02294-03 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Antidepressant Pharmacology of the Rodent Circadian System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. Duncan	Research Psychologist	CPB/NIMH
Others:	L. Tamarkin	Research Biologist	CPB/NIMH
	T. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH

COOPERATING UNITS (if any)

P. Sokolove, Professor of Biological Sciences
University of Maryland, Baltimore County (UMBC)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The circadian system in patients with primary affective disorder is disorganized. A major symptom of this disease includes a disturbed activity-rest cycle. Treatments which affect the circadian system may correct a pathological state which underlies depression. Our goal is to understand how antidepressant chemicals alter the dynamics of the mammalian circadian system. Results from these studies will be valuable to understand the mechanism of chemical antidepressant treatments in humans.

Previously we reported that in hamsters, clorgyline, a monoamine oxidase inhibitor with antidepressant properties, increases the period of the circadian clock and decreases the rest component of the daily activity-rest cycle. We also observed that this chemical alters the response of the circadian clock to light. These results indicate that clorgyline exhibits input properties to the circadian clock; the location of its input has been unclear.

Processing of light information to the clock occurs at either the retina or the lateral geniculate nucleus projection to the clock. Results from the past year indicate clorgyline's effects are not mediated via the retinal projection to the clock. Our current hypothesis is that clorgyline alters the hamster circadian system via a lateral geniculate nucleus projection to the circadian clock. We are currently testing this hypothesis.

Project Description:

Disturbances of the activity-rest cycle which accompany depression may be due to dysfunctional biological oscillators which populate the mammalian circadian system. Chemical antidepressants which effect these abnormal oscillations may exert beneficial clinical properties by correcting a pathological circadian process. We are interested in 1) describing the effects and 2) identifying the input pathway, of antidepressant chemicals on the mammalian circadian system.

Methods:

1) Experimental equipment

A description of the facility used to evaluate and monitor the rodent circadian system can be found in project report Z01 MH 02294-01 CP.

2) Antidepressant chemical effects on the central pacemaker's period

We have extended our studies to include treatment with the selective MAOI deprenyl. High dose deprenyl (25 mg kg⁻¹ day⁻¹) was chronically administered via Alzet osmotic mini-pumps. Hamsters were maintained in constant conditions (constant darkness, temperature, ad lib food and water) Wheel-running activity was continuously collected by laboratory computer.

3) Location of chemical input to the central pacemaker

There are four possible input pathways to the circadian pacemaker: 1. the suprachiasmatic nucleus (SCN), 2. the retina and retinal hypothalamic path to the SCN, 3. the lateral geniculate nucleus and its projection to the SCN, and 4. raphe complex and its projection to the SCN. In order to assess the role of the retina in mediating clorgyline's effects on the circadian clock, hamsters received bilateral orbital enucleation and then were treated with chronic clorgyline as described previously (see Z01 MH 02294-02 CP).

Findings to Date:

Previously we observed that chronic clorgyline treatment of Syrian hamsters 1. increased the period of the circadian clock, 2. increased the activity-rest ratio and 3. altered the response of the circadian system to light. Two observations emerge from our current investigations .

First, our studies suggest that chronic, high-dose deprenyl increased the period of the circadian clock similar to chronic clorgyline treatment. These results indicate the circadian effects are probably related to a Type A MAO property rather than a non-pharmacological property of the clorgyline molecule itself.

Second, we observed that bilateral enucleation failed to block the effect of clorgyline on increasing the period of the circadian clock. Therefore, clorgyline appears to be altering the period either at the SCN itself, via the lateral geniculate nucleus (LGN) projection to the SCN, or via the raphe projection to the SCN.

Our current hypothesis is that clorgyline's effect on the hamster circadian system is via the LGN projection to the SCN. Several recent experiments conducted in the U.S., Canada and Holland, support this hypothesis. LGN lesioned hamsters are functionally similar to

1. an increased activity-rest ratio, 2. an increased clock period and 3. an altered response of the circadian system to light.

Significance to Biomedical Research:

Our experiments indicate that the MAOIs clorgyline and deprenyl, when administered at doses which inhibit Type A MAO, alter the period of the circadian pacemaker in Syrian hamsters. These results are consistent with our hypothesis that the antidepressant mechanism of these compounds in humans may include effects on the circadian clock, and that Type A MAO inhibition probably participates in this response.

Pharmacological interventions (chronic clorgyline) and non-pharmacological interventions (light intensity, LGN lesions) which affect the retino-geniculohypothalamic (RGH) tract are similar in several respects. In Syrian hamsters, each of these treatments increase the activity-rest ratio and the period of the central clock. Also, both LGN lesions and chronic clorgyline treatment alter the processing of light signals by the central clock. A neuroanatomical nucleus which regulates the expression of these circadian based processes has been postulated to be abnormally expressed in some depressed patients.

Our current hypotheses are that since LGN lesions alter the activity-rest ratio, as do MAOI and light treatments, a) a dysfunctional LGN may contribute to the sleep disturbance which accompanies primary depression and b) a common mechanism of pharmacological and non-pharmacological (i.e. bright light) treatments of mood disorders may include functionally similar effects on the LGN.

Proposed Course:

During the next year we plan on extending our studies to include tricyclic antidepressants and lithium. These chemicals will be administered chronically to evaluate their effects on the period of the central clock. Second, we plan on measuring the response of chronic clorgyline treated hamsters to constant light in order to more fully evaluate the similarities between clorgyline treatment and LGN lesions. Third, we will determine the circadian system response of LGN lesioned hamsters to chronic clorgyline treatment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02303-02 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Sleep in Psychiatric Illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: W. Mendelson Chief, Section on Sleep Studies CPB/NIMH

COOPERATING UNITS (if any)

Clinical Psychobiology Branch

LAB/BRANCH

Section on Sleep Studies

SECTION

NIMH, NIH, Bethesda, Maryland 20892

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.3

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In a previous study patients with bipolar depression were compared to age and sex matched controls, there were differences in sleep efficiency and total sleep, but no differences in REM latency or power spectra. We have looked at the power spectra of insomniacs and compared it to controls. There were no differences in power spectra of insomniacs when compared to controls. Our previous studies of the frequency analysis of depressed bipolar patients compared to normal controls slowed difference in sleep efficiency and total sleep but no difference in REM latency and power spectra. We look at the power spectra of sleep in insomniacs and compared it with controls. There were no differences found in any aspect of the frequency analysis.

Project Description:

Ten insomniacs and ten age and sex matched controls had sleep studies which were recorded for analysis.

Methods:

Subjects EEG were recorded on FN tape for future analysis. Tapes were played back through a Bruel and Kjaer frequency analyzer which produces plots of power. All non REM epochs were plotted and averaged over the night. The REM epochs were evaluated separately.

Findings to Date:

There were no differences between insomniacs and controls for REM or non REM power spectra.

Proposed Course:

There is evidence that depressives have a high body temperature especially at night. Cooling at night reduces REM and may have an antidepressant effect. We wish to study depressives exposed to different temperature manipulations to see the effect of temperature on sleep in depressives.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02324-02 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuroendocrine Modulation of Cellular Immune Response

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. Tamarkin	Research Biologist	CPB/NIMH
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Others:	G. Paciotti	Biologist	CPB/NIMH
	R. Skwerer	Medical Staff Fellow	CPB/NIMH
	M. Collins	Biologist	CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects
 (b) Human tissues
 (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The focus of this line of investigation is to compare short-term vs long-term neuroendocrine challenges on the proliferation of splenic lymphocytes. This was accomplished by comparing the in vitro proliferative response of lymphocytes to concanavalin A following an in vivo 4 hour isoproterenol treatment compared to a 5 day constant infusion of isoproterenol. Following the short-term isoproterenol treatment splenic lymphocyte proliferation was inhibited compared to controls, while long-term exposure resulted in an increase in the proliferative response. These results were not related to corticosterone levels which were elevated in both the acute and chronic treatments, nor were they related to the presence of the adrenal gland. These studies suggest that splenic lymphocytes become insensitive to the suppressive effect of chronically elevated glucocorticoid.

Project Description:

Factors that induce a stimulation of glucocorticoid secretion have been associated with a suppression of immune function. To test this we have chosen a specific neurochemical challenge, isoproterenol, that has direct sympathetic effects, particularly on the cardiovascular system. A second chemical challenge that was undertaken was an insulin tolerance test, in which blood glucose levels were dramatically reduced. Following either or both treatments the rats were sacrificed and the spleens removed for processing in a lymphocyte transformation test, using concanavalin A as the mitogen. Cellular proliferation was determined by the amount of ^3H -thymidine incorporated in 6 hours.

Methods:

The acute part of this study was previously described. The long-term isoproterenol treatment was accomplished by implanting rats with Alzet minipumps containing isoproterenol. After 5 days of treatment the animals were given an injection of insulin and after 2 hours the animals were sacrificed. Blood was collected for glucose and corticosterone determination. Pineal glands were frozen for subsequent melatonin content analysis and spleens were harvested for lymphocytes. The lymphocyte transformation test has been previously described.

Findings to Date:

Acute *in vivo* treatment with isoproterenol resulted in a suppression in lymphocyte proliferation. However, chronic isoproterenol treatment resulted in increased lymphocyte proliferation compared to controls. Acutely, as well as chronically treated animals had elevated serum glucocorticoid levels and increased pineal content of melatonin. The data clearly indicate that lymphocytes become desensitized to the inhibitory effect of elevated glucocorticoid.

Significance to Biomedical Research:

These data compare the effect of acute versus chronic systemic chemical challenges on the cellular immune response and suggest that long-term challenges are compensated by a resensitization of lymphocytes to mitogen. This may be critical in defining the range of immune responses to physiologic and pathophysiologic events, and these data provide insight into the sensitivity of the immune system to *in vivo* short-term and long-term activation of the hypothalamic-pituitary-adrenal axis.

Proposed Course:

Assessment of the factor(s) that modulates the cellular immune response needs to be investigated. Further exclusion of the adrenal axis involvement will be pursued and the effect of other endogenous molecules will be studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02325-02 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Light and Lymphocyte Activity: Basic and Clinical Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Tamarkin Research Biologist CPB/NIMH

Others: R. Skwerer Medical Staff Fellow CPB/NIMH
 G. Paciotti Biologist CPB/NIMH
 M. Collins Biologist CPB/NIMH
 J. Rhyne-Grey Biologist CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch
 SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.1

PROFESSIONAL:

1.1

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In last year's report we reported that peripheral blood lymphocytes from patients with winter depression had different in vitro responses to mitogen following bright light treatment compared to placebo treatment. This year the same study was repeated with a different group of patients and the observations were the same as that noted in the first study. To gain some further understanding of the mechanism by which light affected the cellular immune response, a group of normal volunteers was exposed to bright light or ordinary light restricted to their eyes. The data from this study were quite clear, showing that light perceived through the eyes is transduced through the central nervous system, impacting on the cellular immune response.

Project Description:

A human physiologic study was undertaken to determine if the changes in peripheral blood lymphocytes observed in patients also occurred in normal volunteers. Also it is possible that the changes observed following light treatment might have been the result of light (uv) acting directly on the skin. To address this, normal volunteers were gowned so that the bright light exposure could only be perceived by their eyes. Blood components, including lymphocytes, were collected and analyzed.

Methods:

Normal volunteers were recruited and were asked to sit in front of a bank of bright lights (approximately 3000 lux). The subjects were gowned so that the light would be restricted to their eyes. This study was conducted twice, once where treatment occurred in the home and once where it was administered on our clinical research ward. Blood samples were taken before treatment, one day after treatment, and one week after treatment. Peripheral blood lymphocytes were isolated for mitogen testing and the plasma was stored for the analysis of cortisol and antibody titers.

Findings to Date:

Of the parameters studied, peripheral blood lymphocytes were consistently and significantly stimulated after one week of bright light treatment. This occurred for both mitogens and suggests that the cellular immune system can be affected by light perceived through the eyes. The results of this study are being written and will be presented for peer review.

Significance to Biomedical Research:

This study clearly demonstrates the range of physiologic responses of the cellular immune system to environmental light and indicates that this system is responsive to changes in the environment that can be transduced through the central nervous system to impact on the internal milieu. This study advances our knowledge by suggesting one environmental factor, light processed through the central nervous system, that may impact on the immune system's ability to ward off various infectious diseases.

Proposed course:

More sophisticated analysis of the specific immune cells affected by bright light exposure will be the focus of future studies. This will be accomplished by using monoclonal antibodies that can identify specific immunologic cell sub-types and determining their distribution by flow cytometry. This technology is readily available for human lymphocytes and for mouse lymphocytes. To pursue the latter, we need to determine if mice also respond to bright light treatment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02326-02 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Modulation of the Cellular Immune Response

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. Tamarkin	Research Biologist	CPB/NIMH
Others:	M. Collins	Biologist	CPB/NIMH
	R. Skwerer	Medical Staff Fellow	CPB/NIMH
	G. Paciotti	Biologist	CPB/NIMH

COOPERATING UNITS (if any)

S. Suomi, Laboratory of Comparative Ethology, NICHD, NIH

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.2

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Young rhesus monkeys were placed into groups of 4 by slowly introducing them to less and less controlled environments. Blood samples were taken from awake animals in under 5 minutes and peripheral blood lymphocytes were isolated and cultured. The data from three groups of 4 indicate that the most dominant animal in the group has suppressive effect on the ability of lymphocytes to proliferate in response to mitogen in vitro.

Project Description:

Young rhesus monkeys were introduced to a novel cage setting. Dominance was determined and blood samples were taken from awake animals. The goal of the study was to determine if behavioral challenges rapidly affect the proliferative response of lymphocytes.

Methods:

Unchanged from that outline last year.

Findings to Date:

The behaviorally dominant animal consistently had a more robust lymphocyte proliferation response, while the more submissive animals had lymphocyte proliferation responses that were clearly compromised and suppressed.

Significance to Biomedical Research:

These data suggest that social behavior impacts on the immune system's ability to recognize an antigenic challenge. It is not clear at this time what the impact is on susceptibility to systemic infections, and this needs to be the focus of future research.

Proposed course:

Further characterization of immune cells by flow cytometry may provide us with new insight on the specific cell types affected by behavioral challenges. Also the time course of these changes is critical in the evaluation of the range of responsiveness.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02327-02 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Direct effect of Lymphokines on Cultured Human Breast Cancer Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Tamarkin Research Biologist CPB/NIMH

Others: G. Paciotti Biologist CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Interleukin 1 and interleukin 2 are products of the immune system whose action has been almost exclusively studied in the modulation of immune cell growth. Essentially these molecules are considered paracrine hormones. Our hypothesis is that these proteins may be endocrine hormones, whose action may be on cells of non-immune origin. We have seen that both IL-1 and IL-2 affect human breast cancer cell growth and further that this action occurs on hormone-dependent breast cancer cells, but not on hormone-independent breast cancers. These studies have been conducted in vitro using ³H-thymidine incorporation as an index of proliferation or actual cell number. Additionally, an IL-1 receptor has been characterized on MCF-7 breast cancer cells. In vivo IL-2 has been shown to suppress tumor cell growth using athymic nude mice implanted with tumor cells and IL-2 as the model system.

Project Description:

In vitro: In last year's report 3H-thymidine was the only measure of cell growth described. This method is a standard technique, however, it is possible that it may not be valid and other measures of cell growth should be used to confirm 3H-thymidine observations. The unambiguous experiment to perform is to plate cells and count them at various days following the initiation of treatment. By following cell growth a clear effect of both IL-1 and IL-2 on MCF-7 cells was demonstrated. It should be noted that these cells are hormone dependent cells, having a full complement of hormone receptors and are estrogen dependent when implanted *in vivo*. To test whether or not the effect of IL-1 and IL-2 is related to the hormone-dependency of the cell line, two other hormone independent breast cancer cell lines were used in parallel experiments.

Current scientific knowledge concerning the mechanism of action of the proteins is that they have specific cell surface receptors. Using the MCF-7 cells an IL-1 receptor has been demonstrated. This receptor is not measurable on the hormone independent cells.

In vivo: A critical test of the hypothesis that IL-2 may be acting as a hormone is to determine if it has any growth inhibiting effect in an animal. The model system is the athymic nude mouse. The strain is immunocompromised in that these animals have few T-cells, which are not responsive to IL-2. Measurable tumors were observed by implanting ovariectomized nude mice with MCF-7 cells and estrogen pellets. Once tumors were observed, the animals were then implanted with IL-2 pellets.

Methods:

MCF-7 or ZR-75 breast cancer cells have both been shown to be hormone dependent tumor cell lines. MDA-231 and HS-578-T have been shown to be hormone independent breast tumor cell lines. Cells were plated, allowed to attach to the plates, and then treated with either lymphokine. Individual wells were harvested every second or third day for 12 days and the cells were counted in a particle counter. For the characterization of an IL-1 receptor on MCF-7 cells, iodinated IL-1 was incubated with plated cells. Cells were washed, detached and radioactivity determined. This study was done four different ways: 1) hot only, 2) one dose of hot and competing doses of cold, 3) various doses of hot and 200-fold excess of cold for each dose, and 4) cross-linking of uncompleted and competed hot IL-1 followed by PAGE-SDS analysis.

Findings to Date:

Both IL-1 and IL-2 affected hormone dependent breast cancer cell growth. This was observed using either 3H-thymidine as an index of proliferation or cell number. Similar effects were not noted for the hormone independent breast cancer cell lines. An IL-1 receptor has been characterized on MCF-7 cells that has a Kd similar to that shown for T-cells. *In vivo* tumor cell size was markedly inhibited by the IL-2 pellet compared to controls and this was not associated with an increase in T-cell function nor with an increase in NK-cell activity. IL-2 had no effect on the rapid growth of the hormone independent cells (MDA).

Significance to Biomedical Research:

These data provide more evidence that these lymphokines may be acting as hormones. The regulation of lymphokine secretion is not entirely clear and factors impinging on the CNS may directly or indirectly affect their secretion, which may have direct bearing on the etiology of specific systemic diseases, such as cancers.

Proposed course:

Further characterization of the IL-1 receptor and characterization of the IL-2 receptor are essential. The possible mechanism by which these lymphokines affect these cells may be through a common growth factor, such as transforming growth factor- β , and the relationship between lymphokines and TGF- β needs to be investigated. Finally, these lymphokines may be affecting breast cancer cell cycles and characterization of cell cycles using flow cytometry provides a new model for evaluating the effect of these lymphokines on these cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02328-02 CP

PERIOD COVERED
 October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
 Direct effects of IL-2 on Cultured Anterior Pituitaries

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. Tamarkin	Research Biologist	CPB/NIMH
Others:	G. Paciotti	Biologist	CPB/NIMH
	M. Collins	Biologist	CPB/NIMH
	K. Nyhus	Summer Student	CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
 Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION
 NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 1.0	PROFESSIONAL: 1.0	OTHER:
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CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The possibility that lymphokines affect other hormone dependent tumors, particularly tumor cells from the pituitary is the focus of this study. AtT-20 is a mouse tumor cell line that secretes ACTH and we have seen that the growth of these cells and the secretion of ACTH is affected by both IL-1 and IL-2. When tumor cell growth is inhibited, ACTH secretion per cell is increased. Conversely, when tumor cell growth is increased ACTH secretion per cell is diminished.

Project Description:

AtT-20 cells were grown in tissue culture plates and treated with various doses of IL-1 or IL-2. Cell growth was determined and the media was harvested for the determination of ACTH concentration.

Methods:

Cells were plated and harvested for cell counting or for determination of ACTH concentration in the media. This was done by radioimmunoassay for ACTH.

Findings to Date:

Data indicate that ACTH secretion into the media is increased when cell growth is inhibited, and conversely, when cell growth is increased ACTH secretion is diminished.

Significance to Biomedical Research:

These data suggest that the effect of IL-1 or IL-2 is not restricted to breast cancers and may include a variety of hormone dependent cells. The possibility exists that these lymphokines play a role in cell differentiation and are critical for maintenance of the physiologic function of the cell.

Proposed course:

Evaluation of the genetic message for ACTH is being initiated and determination of changes in the cell cycle with flow cytometry will also be undertaken in the next year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02382-01 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

An Animal Model for Human Sleep Apnea

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W.B. Mendelson	Chief, Section on Sleep Studies	CPB/NIMH
Others:	J.V. Martin	Chemist	CPB/NIMH
	R. Wagner	Guest Worker	CPB/NIMH

COOPERATING UNITS (if any)

S.I. Rapoport Chief, Laboratory of Neurosciences, NIA

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Section on Sleep Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.9

PROFESSIONAL:

0.5

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Using a non-invasive technique which measures respiration as a function of chest and abdominal movements, it was found that adult rats have periodic cessations in breathing analogous to human apneas. In preliminary studies in Fischer-344 rats apneas tended to be more frequent in 22-month-old than in 3-month-old rats. As in human apnea, apneas in Sprague-Dawley rats were significantly more frequent and longer in duration in REM sleep than in nonREM sleep or waking.

The project was terminated in July, 1987 when the investigators in the Section on Sleep Studies left NIH.

Project Description:

The purpose of this study was to characterize spontaneously occurring cessations in breathing in rats as an animal model of human sleep apnea. This model should be useful in studies of the neural mechanisms underlying sleep apnea.

Methods:

Respiration was determined by a specially modified Columbus Instrument Respiration Monitor Model RM 80 (Columbus Instrument Co., Columbus, Ohio). Rate and amplitude of respiration are derived from the dynamic pressure change in a closed chamber due to movement of the chest and abdominal wall of an unrestrained rat. In preliminary studies, the respiration of intact 3-month-old and 22-month-old male Fischer-344 rats was characterized in 30 minute tests. In further studies, male Sprague-Dawley rats were implanted with electrodes on the skull and in the neck musculature, and after the rats recovered from surgery, 6-hour EEG and EMG recordings were made along with the record of respiration (See Project Z01 MH 02225-01-CP). The EEG records were scored for waking, nonREM sleep, or REM sleep and the occurrence of apneas lasting 2 seconds or longer was determined within each of these defined stages of consciousness.

Findings to Date:

The respiratory rate of 22-month-old Fischer rats was found to be lower ($p < 0.006$) and the tidal volume higher ($p < 0.02$) than in 3-month-old Fischer rats. Rats of both ages had multiple cessations of respiratory effort lasting 2 seconds or longer. The number of apneas during the 30 minute test tended to be higher in the 22-month-old rats (3.9 ± 0.9 events; mean \pm S.E.M.) than in the 3-month-old rats (2.5 ± 0.5 events) but this difference did not reach statistical significance ($p < 0.07$).

In a further six hour study in Sprague-Dawley rats, the apneas were characterized with greater resolution and correlated with the EEG stage of consciousness. Cessations of breathing preceded by large inspiratory movements were noted. These events were presumed to be due to lowered $p\text{CO}_2$ after a deep breath and were not considered further. More importantly, all of the rats showed instances of cessations of breathing which were not preceded by unusually large inspirations (19 ± 1.5 events/6 hours). These apneas varied significantly ($p < 0.03$) in occurrence during the 6-hour test according to EEG stage, with a predominance in REM sleep (waking = 3.5 ± 1.0 events; nonREM sleep = 4.7 ± 1.2 events; REM sleep = 8.4 ± 2.1 events). The duration of the apneas also varied significantly ($p < 0.006$) according to EEG stage (waking = 2.5 ± 0.1 sec/event; nonREM sleep = 2.1 ± 0.1 sec/event; REM sleep = 2.8 ± 0.1 sec/event). A bradycardia was apparent in the EMG recording during many apneas.

Significance to Biomedical Research:

Sleep apnea syndrome is the most common diagnosis made in patients presenting with excessive sleepiness at sleep disorder centers. Its complications, which include pulmonary hypertension, cor pulmonale, systemic hypertension, and psychiatric symptomatology, make it a matter of substantial public health concern. This research is the first development of an adult animal model for sleep apnea. Such a model should be useful in elucidation of the neural mechanisms responsible for

sleep apnea. In addition, clinically oriented programs could benefit from an animal model of sleep apnea, in for example, the evaluation of drugs which have the potential of exacerbating the syndrome.

Publication:

Mendelson, WB, Martin, JV, Perlis, M, Giesen, H, Wagner, R. and Rapaport, S.I.: Apneas during sleep in adult rats. Sleep, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02383-01 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects on Sleep of a Microinjection of Triazolam to Discrete Brain Loci

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W.B. Mendelson	Chief, Section on Sleep Studies	CPB/NIMH
Others:	J.V. Martin	Chemist	CPB/NIMH
	H. Stevens	Psychologist	CPB/NIMH
	R. Wagner	Guest Worker	CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

Section on Sleep Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20982

TOTAL MAN-YEARS:

1.4

PROFESSIONAL:

0.5

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

As part of a systematic investigation of the possible brain sites at which benzodiazepines (BZ's) alter sleep, 0.5 µg of triazolam was injected into the dorsal raphe nucleus (DRN) of rats. Injections of triazolam to the DRN significantly increased sleep latency and decreased nonREM sleep as compared to injections of vehicle to the DRN or injections of triazolam to brain areas near but outside the DRN.

This project was discontinued following departure of the investigators from NIMH in July, 1987.

Project Description:

Although the hypnotic properties of parentally administered BZ's are well described, the neuroanatomical sites responsible for the hypnotic action of these drugs are unknown. While our previous work has provided evidence that the sleep-inducing effects of BZ's are mediated through an action at the BZ receptor, these receptors are widely distributed throughout the brain. Through lesion and electrical stimulation studies, numerous brain sites are implicated as functional centers critical to regulation of specific aspects of sleep. The purpose of this project is to examine the effects on sleep of the application of a BZ to a variety of brain areas, including those sites classically associated with sleep regulation. In this way, we hope to characterize the neuroanatomical sites of action of BZ's in relation to sleep.

Methods:

Adult male Sprague-Dawley rats are stereotaxically implanted with a 24 ga stainless steel cannula whose tip rests 1.0 mm dorsal to the anatomical site of interest. During the same procedure, stainless steel screws are implanted to serve as dural EEG electrodes, and teflon-coated stainless steel wires are inserted into the nuchal musculature for EMG recording. After a one week recovery period, a 31 ga stainless steel cannula is lowered through the outer cannula, extending 1.0 mm beyond its tip, and vehicle or 0.5 µg triazolam in a volume of 0.5 µl is administered at a rate of 0.21 µl/min. Immediately after injection of vehicle or drug, an eight hour sleep recording is performed. One week later, the alternative injection of vehicle or triazolam is given in the same manner as the first injection, and a second EEG recording is performed. Finally, the rats are injected with 0.1 µl of methylene blue dye, perfused, and histological localization of the injection site is determined.

Findings to Date:

Triazolam injections into the DRN were found to have an alerting effect. Sleep latency was increased from control values of 20.0 ± 3.8 min (mean \pm S.E.M.) to 41.4 ± 4.2 min ($n = 10$; $p < 0.002$). In contrast there was no significant effect of triazolam on sleep latency when injected to sites near but outside of the DRN (vehicle = 26.9 ± 3.6 min; triazolam = 23.8 ± 5.6 min; $n = 6$).

Due to the proximity of the DRN to the cerebral aqueduct, the possibility existed that the drug diffused into the cerebrospinal fluid and exerted its effects at a site remote to the DRN. For this reason, we carried out a sleep study after injection of vehicle, 0.5 µg or 1.0 µg of triazolam to the lateral ventricle of 10 rats. Sleep latencies for these conditions were 19.6 ± 1.6 min, 18.4 ± 4.0 min and 18.6 ± 3.2 min, respectively (difference not significant). The dose of triazolam employed here, then, did not alter sleep induction when administered intraventricularly.

Injection of triazolam to the DRN also significantly decreased total sleep ($p < 0.01$) and nonREM sleep ($p < 0.02$) and increased intermittent waking ($p < 0.03$), without a significant effect on REM sleep. A drug x time period interaction ($p < 0.01$) indicated that the effect on total sleep was greatest during the first two hours after administration of the drugs.

Triazolam, therefore, has a potent but transient alerting effect when injected to the DRN.

Significance to Biomedical Research:

The seemingly paradoxical finding that a drug which induces sleep when given systemically may produce arousal when given locally may have a variety of implications for sleep research. It is known that a GABAergic influence on the DRN has an inhibitory effect on cell firing in the DRN and on serotonin turnover in these cells. Since BZ's increase the affinity of GABA for its

Project Description:

Although the hypnotic properties of parentally administered BZ's are well described, the neuroanatomical sites responsible for the hypnotic action of these drugs are unknown. While our previous work has provided evidence that the sleep-inducing effects of BZ's are mediated through an action at the BZ receptor, these receptors are widely distributed throughout the brain. Through lesion and electrical stimulation studies, numerous brain sites are implicated as functional centers critical to regulation of specific aspects of sleep. The purpose of this project is to examine the effects on sleep of the application of a BZ to a variety of brain areas, including those sites classically associated with sleep regulation. In this way, we hope to characterize the neuro-anatomical sites of action of BZ's in relation to sleep.

Methods:

Adult male Sprague-Dawley rats are stereotaxically implanted with a 24 ga stainless steel cannula whose tip rests 1.0 mm dorsal to the anatomical site of interest. During the same procedure, stainless steel screws are implanted to serve as dural EEG electrodes, and teflon-coated stainless steel wires are inserted into the nuchal musculature for EMG recording. After a one week recovery period, a 31 ga stainless steel cannula is lowered through the outer cannula, extending 1.0 mm beyond its tip, and vehicle or 0.5 μg triazolam in a volume of 0.5 μl is administered at a rate of 0.21 $\mu\text{l}/\text{min}$. Immediately after injection of vehicle or drug, an eight hour sleep recording is performed. One week later, the alternative injection of vehicle or triazolam is given in the same manner as the first injection, and a second EEG recording is performed. Finally, the rats are injected with 0.1 μl of methylene blue dye, perfused, and histological localization of the injection site is determined.

Findings to Date:

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Injection of triazolam to the DRN also significantly decreased total sleep ($p < 0.01$) and nonREM sleep ($p < 0.02$) and increased intermittent waking ($p < 0.03$), without a significant effect on REM sleep. A drug \times time period interaction ($p < 0.01$) indicated that the effect on total sleep was greatest during the first two hours after administration of the drugs.

Triazolam, therefore, has a potent but transient alerting effect when injected to the DRN.

Significance to Biomedical Research:

The seemingly paradoxical finding that a drug which induces sleep when given systemically may produce arousal when given locally may have a variety of implications for sleep research. It is

recognition site, it seems likely that local administration of a BZ will suppress dorsal raphe cell function through a potentiation of the effects of GABA. The arousing effects of a possible inhibition of the DRN by a BZ are in keeping with studies which have demonstrated an acute waking effect of lesions of the DRN. These studies support the idea that the DRN is a brain site important to the initiation of sleep, albeit not the site by which BZ's have their hypnotic effects when given parenterally. The demonstration of a brain site which causes arousal in response to BZ's might imply that not all of the BZ receptors in the central nervous system have a role in mediating the hypnotic effects of BZ's. There may be a functional division of BZ receptors according to neuroanatomical organization. Examination of the effects on sleep of microinjection of BZ to a variety of brain nuclei will be necessary to clearly characterize the neuroanatomical sites responsible for the hypnotic effects of these widely-prescribed drugs.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02402-01 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Causes and Treatment of Summer Depression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T. A. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH
Others:	N. E. Rosenthal	Chief, Unit on Outpatient Studies	CPB/NIMH
	P. Schulz	Social Worker	CPB/NIMH
	S. Kasper	Visiting Scientist	CPB/NIMH
	K. Kelly	Medical Staff Fellow	CPB/NIMH
	J.R. Joseph-Vanderpool	Medical Staff Fellow	CPB/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Seasonal patterns of affective illness are of particular interest because they suggest that environmental factors may be capable of causing and terminating affective episodes. We recently identified a group of patients who become depressed in summer and normal, or hypomanic, in winter. This pattern is opposite to one we previously described in patients who become depressed in winter, and who respond to treatment with bright artificial light. Approximately 30 summer depressives have been studied. Symptom profiles of winter and summer depression are similar in most respects, except that winter depressives usually oversleep and overeat, while summer depressives sleep less and lose weight. Both types of depression are much more common in women than in men. We tested the hypothesis that seasonal changes in light or temperature might cause summer depression, in a balanced randomization crossover study comparing possible therapeutic effects of darkness and cold. The results of the study were encouraging, in that patients improved significantly after both types of treatment. However, the results did not enable us to distinguish between the respective roles of light and temperature. We are following up with a complementary study of the capacity of heat and light to induce depressive symptoms in this population. Thyroid hormones decreased when patients became depressed in the summer. Further research is necessary to determine whether these changes are responsible for summer depressions. In nine patients, positron emission tomography (PET) scans were obtained during their summer depressions. These will be repeated during fall/winter remissions for comparison.

Project Description:

Seasonal patterns of occurrence of some types of affective illness suggest that physical environmental factors, such as temperature or light, may be capable of causing and terminating episodes of depression and mania. Indeed this has been found to be the case with winter depression, which responds to treatment with light, and which therefore may be caused by cyclic seasonal reductions in natural light. While most of our research on seasonal affective disorder has focused on winter depression, we recently identified and have begun to study a group of patients with recurrent summer depressions. Our aim was to validate patients histories by prospectively ascertaining that they become depressed in the summer and to investigate the possible role of changes in light and/or temperature as causes, and potential treatments, of summer depression.

Methods:

Patients were recruited by referral by clinicians familiar with our studies of seasonal depression or by descriptions in the media of our research program. Patients' diagnoses were based on the Revised Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-III-R) using data obtained with the Structured Clinical Interview (SCID). Their clinical state was assessed with the Hamilton Depression Rating Scale (HDRS), the Weekly Mood Inventory (WMI), and visual analogue rating scales (VAS).

Thyroid axis hormones (known to be influenced by temperature changes) were measured in the spring (before the onset of depression) and summer (during depression).

Positron Emission Tomography (PET) scans were obtained during summer depressions and after cold treatment.

Patients were exposed to two treatment conditions: 1) isolation from bright light (with special neutral density glasses) and exposure to darkness, and 2) isolation from heat (with airconditioning) and exposure to 40° F. The treatments were carried out during two different five day periods, with time between the periods to allow for relapse if patients improved. Dark and cold exposures lasted twenty minutes and were repeated four times a day. For an additional twenty minutes four times a day patients were exposed to outdoor heat on the dark condition and outdoor light on the cold condition. Clinical state before and after treatment and after withdrawal of treatment was assessed with blind raters using the HDRS and by the patients using the VAS. Patients' expectations of the two treatments were assessed before the treatments began.

Findings to Date:

Thirty patients with a history of summer depression were diagnosed with the SCID/DSM-III-R. Most had a bipolar II pattern of illness, with major depression in the summer and hypomania in the winter. The most frequent symptoms of depression were lethargy, social withdrawal, loss of interest, insomnia, appetite and weight loss, loss of interest in sex, sadness. Less frequent were guilt, hopelessness and suicidal thoughts.

Twenty-two patients were followed into the summer season and of these all but two became significantly depressed (HDRS score ≥ 15), as predicted by their histories.

Thyroid hormone levels decreased in the summer compared with the previous spring.

PET scans are not yet analyzed.

Eight patients agreed to participate in the experimental cold versus dark treatment study. Patients significantly improved after both types of treatment with approximately 50% reductions in HRSD scores. There are several possible explanations for this result.

- a. Both temperature and light are capable of regulating mood
- b. The effects of temperature and light were confounded in the study
- c. Some other factor(s) that were not controlled in the study (e.g., hospitalization) were responsible for the improvements.

The difficulty in interpreting this study can only be resolved by additional research. Many unsystematic retrospective and prospective observations of the course of illness in these patients suggest that changes in temperature (and humidity) play a major role in the occurrence of summer depressions. However, these clinical insights must be subject to experimental verification.

Significance to Biomedical Research:

Studies of seasonal depression provide a unique opportunity to explore the possible role of physical environmental factors, such as light and temperature, as causes of and treatments for depression. This type of research has already led to the development of a new type of antidepressant treatment, phototherapy, for depression, and has stimulated a series of basic studies of biological effects of ocularly mediated light as possible mechanisms. This research, in turn, has led to the discovery of eye-mediated effects of light on immune function, effects that may prove to be relevant to a variety of seasonally occurring infectious and autoimmune diseases. In the same way, proof of effects of temperature on clinical state could be expected to lead to additional new types of treatment for depression and mania, and to new knowledge about the biological effects of environmental temperature.

Proposed Course:

To further investigate the possible role of temperature and/or light as causes of depression and mania, patients will be exposed to bright light and to heat during seasons other than summer. The finding that thyroid hormone levels decline as patients become depressed in the summer will be followed up by more detailed investigations of the thyroid axis in these patients through the course of the year.

Publications:

Wehr TA, Sack DA, Rosenthal NE: Seasonal affective disorder with summer depression and winter hypomania. *American Journal of Psychiatry*, in press.

Wehr TA, Sack DA, Rosenthal NE: Environmental and Behavioral Influences on Affective Illness. *Acta Psychiatrica Scandinavica*, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02403-01 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Action of Melatonin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. Tamarkin	Research Biologist	CPB/NIMH
Others:	G. Paciotti	Biologist	CPB/NIMH
	M. Collins	Biologist	CPB/NIMH
	J. Rhyne-Grey	Biologist	CPB/NIMH

COOPERATING UNITS (if any)

Steven Reppert, Associate Professor, Children's Service, Massachusetts General Hospital
 Arayan Namboodiri, Assistant Professor, Department of Biology, Georgetown University

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The melatonin analog, iodo-melatonin, may provide us with an ideal agonist to identify, characterize, and localize the melatonin receptor. We have made radioactive and "cold" iodo-melatonin. This product has been characterized by HPLC and the "cold" iodo-melatonin has been shown to be biologically active. Preliminary data indicate that melatonin can displace iodo-melatonin binding to a crude supernatant of hamster brain, but no binding was observed with similarly prepared rat brain homogenate.

Project Description:

For testing the biological activity of iodo-melatonin, Siberian hamsters were chosen as the model system. Previous studies have shown that melatonin infusions of specific durations will signal gonadal development in the offspring or inhibit it. Exploiting this rapid response (a 4 day prenatal infusion and 4 week postpartum observation before sacrifice), prenatal infusion of iodo-melatonin was done on pregnant Siberian hamsters. For testing the binding activity ^{125}I -iodo-melatonin will be prepared and incubated with or without cold melatonin to investigate displaceable binding in membrane and/or cytosol fractions from a number of organs (including specific brain regions).

Methods:

Pregnant Siberian hamsters are infused with iodo-melatonin for 10 hours or 6 hours per day on days 16 to 20 of pregnancy. The pups are maintained in a LD 14:10 lighting cycle until 30 days of age. Animals are sacrificed and testes weights are recorded. For the characterization of melatonin receptors ^{125}I -iodo-melatonin is prepared by iodination with iodogen (a similar synthetic procedure is also done to prepare the cold iodo-melatonin) and the material separated using HPLC. Crude tissue homogenates are prepared from rats or hamsters and the assay is incubated in the cold for 4 hours. The separation of bound from free is accomplished by using glass filters pre-soaked in polyethylenimine.

Findings to Date:

Pups from pregnant dames infused with iodo-melatonin for 10 hours had large testes, similar to that seen with a melatonin infusion. Pups from dames infused with iodo-melatonin for 6 hours had widely varied testes weights, different from that seen with melatonin. A melatonin infusion for 6 hours results in consistently small testes in the pups. Displaceable binding was observed in a crude brain preparation from the hamster, but not from the rat. Specific binding was also observed in a homogenate from hamster pituitary and ovary.

Significance to Biomedical Research:

The *in vivo* data suggest that iodo-melatonin is a melatonin agonists whose affinity for the melatonin receptor is greater than that of melatonin. These data suggest that this ligand will be ideal in the characterization of the site of action of the pineal hormone melatonin. The preliminary binding data also argue that melatonin may be active in the hamster, but is not active in the rat.

Proposed course:

For the *in vivo* study a clearance experiment would help us determine if iodo-melatonin is metabolized to another active compound. Second, *in vivo* injection of ^{125}I -iodo-melatonin will help us determine if specific uptake occurs in specific tissues. *In vitro*, further characterization of a melatonin receptor and its subcellular localization needs to be done. Second, CNS autoradiography needs to be done to determine if specific brain regions have melatonin receptors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02405-01 CP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Chemical Antidepressant Effects on Body Mass and Body Composition in Hamsters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. Duncan	Research Psychologist	CPB/NIMH
Others:	T. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH

COOPERATING UNITS (if any)

T.J. Bartness, Senior Research Associate,
 Worcester Foundation Experimental Biology

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.5

PROFESSIONAL:

.25

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects
 (a1) Minors
 (a2) Interviews
 (b) Human tissues
 (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In humans, one of the side effects of chronic chemical antidepressant treatment is a change in body mass. The mechanism of these induced changes is not clear but may include alterations in metabolic rate, as well as effects on specific body components such as body lipid, protein or water. We have previously noted chemical antidepressant induced alterations in the body mass of Syrian hamsters being treated chronically with the monoamine oxidase inhibitor (MAOI) clorgyline. This project was undertaken to more formally explore the behavioral and physiological mechanisms responsible for these alterations in Syrian hamsters.

We have observed that initial clorgyline treatment decreases food intake. During chronic drug administration, clorgyline administration reduces the rate of weight gain although the level of food intake is not different from control. Thus, drug treated hamsters consume more food per unit of body mass than do control animals, i.e. they exhibit a negative energy balance relative to control animals.

Analysis of the carcass composition of clorgyline treated hamsters indicates that drug induced changes are due to a decrease in body lipid content, rather than protein or water. Since seasonal changes in body mass are due to fluctuations in body lipid content, our results indicate that clorgyline may be affecting the same photoperiodic process which is responsible for photoperiodic-induced changes of body mass in the Syrian hamster.

Project Description:

Antidepressant chemicals produce changes in body mass when administered chronically to humans. These side-effects may be due uniform changes in body component composition or to selective effects on body lipid, protein or water content. Our objectives were to describe the effects of chronic antidepressant drug treatment on body mass in Syrian hamsters, and then to identify the specific body components affected by the drug treatment.

Methods:

1) Treatment

Hamsters were treated with the MAOI clorgyline ($2 \text{ mg kg}^{-1} \text{ day}^{-1}$) administered via osmotic minipumps implanted subcutaneously. Treatment continued for at least two months.

2) Clorgyline's effects on body mass and food intake in LD 14.5:9.5

Hamsters were group-housed in LD 14.5:9.5. Food intake of group-housed and body mass of individual hamsters were measured at two-three day intervals.

3) Clorgyline's effects on body mass and carcass composition in constant darkness (DD).

Individually housed hamsters with running-wheel access were maintained in DD and were weighed at least once per week. After two-months, hamsters were sacrificed and carcass composition determined for lipid, protein and water content.

Findings to Date:

We have observed two effects of chronic clorgyline on Syrian hamster body mass. First, the total body mass of clorgyline treated hamsters was significantly less than the total body mass of saline treated hamsters. This change in body mass was not due to a chronic decrease in food intake. Clorgyline treated hamsters consumed more food per unit body mass than the control hamsters and were therefore exhibiting a negative energy balance compared to control hamsters. Second, analysis of carcass composition indicated that the change in body mass was not due to a uniform change in protein, lipid and water compartments, but specifically due to a decrease in body lipid content.

Significance to Biomedical Research:

Chemical antidepressants often induce undesirable changes in body mass in patients being treated for depression. The mechanism(s) underlying these changes probably include drug-induced effects in metabolic rate and differential effects on body compartment composition.

The physiological and metabolic effects of these compounds on body mass are not understood and it would be useful develop an animal model for careful examination of these drug effects.

We have observed alterations of body mass and body lipid composition in Syrian hamsters treated with chronic clorgyline. The Syrian hamster is a photoperiodic species which regulates seasonal changes in body mass by altering body lipid content. These data indicate that clorgyline may alter body mass in humans by affecting a circannual or photoperiodic process in weight regulation.

Proposed Course:

During the next year, our studies will be directed in three areas. The first area of interest is to examine the effects of tricyclic compounds on body mass and composition. The second area to examine is the effect of antidepressant compounds on another seasonal rodent species, the Siberian hamster, whose body mass response to chronic antidepressant treatment may more closely resemble the human response. Third, we want investigate the effects of these compounds on metabolic rate in order to understand the clorgyline-induced negative energy balance observed in Syrian hamsters.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00274-13 LCS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Methods of Ionization in Mass Spectrometry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH
 Others: Jeffrey P. Honovich, NRC Research Associate, SAB, LCS, NIMH
 Tao-Chin Wang, Fogarty Fellow, IRP, NINCDS

COOPERATING UNITS (if any)

Department of Pharmacology, George Washington University, Washington, DC
 Oak Ridge National Laboratory, Analytical Chemistry Division, Oak Ridge, TN

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

1.5

OTHER:

2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mechanical and software improvements have been installed and tested on the dual cell Fourier transform ion cyclotron resonance spectrometer (FT-ICR) which have corrected many of the previously encountered problems. Laser desorption of polyethyleneglycol mixtures has been used to optimize ion optics and test ion trapping and transfer. Experiments to optimize sample sensitivity and detectability of neuropeptides are in progress.

Collaborative studies on an organic ion microprobe to image the distribution of organic substances in tissue slices are continuing at Oak Ridge National Laboratory. Collaborative studies on element and nuclide selective analyses using a microwave reaction interface for gas chromatography-mass spectrometry are in progress at George Washington University.

Other Professional Personnel Engaged on Project:

Fred P. Abramson	Guest Worker	Professor, Department of Pharmacology, G.W. Univ. Washington, DC
Peter J. Todd	Collaborator	Research Scientist, Oak Ridge Nat'l Lab., Oak Ridge, TN

Project Description

Objective:

Improvement in the specificity and detectability of complex organic compounds in biological matrices requires new developments in mass spectrometric instrumentation. Surface ionization techniques (laser desorption, fast atom bombardment) are being explored for the trace analysis of neuropeptides, neurohormones, and drug metabolites. Fourier transform ion cyclotron resonance spectroscopy (FT-ICR) is being explored as a high sensitivity and high performance mass analyzer. The objective of these studies is to develop and utilize analytical procedures to resolve problems which cannot be solved with conventional instrumentation.

Methods Employed:

Mass spectrometric instrumentation is designed, built, modified, or purchased as required to meet the above objectives.

Major Findings:

During the past year major instrumentation changes were implemented on the FT-ICR, correcting many of the previously detailed deficiencies. In order to optimize laser desorption (LD)-FT-ICR, the behavior of electron impact produced ions was investigated in the dual cell ICR. Ions produced by electron impact in the high pressure source cell can be transferred through a conductance limit to a low pressure analyzer cell by grounding the conductance limit for controlled time intervals. Mechanical alignment of the dual cell assembly in the magnetic field was optimized by observing ion transfer efficiency during ionization; this procedure influenced transfer efficiency by two orders of magnitude. Alternatively, packets of ions stored in the source cell after ionization could be transferred to the analyzer by grounding the conductance limit for a specific time, thus simulating LD conditions. There is a mass dependent transfer frequency because packets of ions of the same mass move with a characteristic speed. At low pressures (10^{-8} - 10^{-7} torr) ion transfer was very efficient (> 90%), but at higher pressures the transfer efficiency was very poor. Upon LD, high mass ions were transferred to the analyzer region in a time-dependent fashion as was observed in EI, although LD produces ions with a wider kinetic energy distribution than EI. LD-FT-ICR analyses of various polyethyleneglycol mixtures demonstrated that (1) useful spectra could be obtained on mixtures representing a wide range of molecular weights without discrimination; (2) summed spectra are reproducible and compound dependent; (3) and that high resolution mass analysis following LD may be possible although not routine.

The transfer of the organic ion microprobe analyzer previously developed as a joint effort between NHLBI and BEIB to Oak Ridge National Laboratory was completed. Dr. Todd has restructured and reconfigured this instrument in order to improve its performance, and is in the process of designing new ion lenses to improve sensitivity.

The microwave discharge interface designed and built in SAB/LCS continues to undergo evaluation and modification at George Washington University. Planned studies with MPTP metabolites were not performed because ^{14}C -MPTP metabolites were identified by other means.

Significance to Biomedical Research:

Structure elucidation of unknown compounds in complex mixtures, or the specific detection and quantification of known compounds and their isotopic variants remain important areas of biomedical research. Polar, non-volatile compounds are frequently encountered in neurochemistry, and the ionization methods and instrumentation being tested are particularly relevant to the analysis of neuropeptides and neurohormones.

Proposed Course:

Optimization of the LD-FT-ICR for neuropeptides will be pursued, with extensive evaluation of sensitivity parameters. Probe surfaces, materials, and means of sample application and concentration will be evaluated. The ability to measure isotopically-labelled neuropeptides will be studied for determining the feasibility of in vivo turnover studies.

The ion microprobe system will be tested with MPP^+ and MPP^+ analogues for its suitability for spatial analysis. The microwave discharge interface will be tested with metabolites of labelled neurotoxins.

New instrumentation for routine high sensitivity GC-MS and fast atom bombardment-tandem MS will be acquired and installed.

Publications:

Abramson, F.P. and Markey, S.P.: Mass spectrometric analysis of sulfur in microgram quantities of biological macromolecules using a reaction interface. *Biomed. Environ. Mass Spectrom.* 13: 411-415, 1986.

Markey, S.P.: Mass spectrometry: Recent developments. *J. Clin. Pharm.* 26:406-411, 1986.

Markey, S.P.: Principles and applications of mass spectrometry in clinical chemistry. Presented at XIII Int. Congress of Clin. Chem., The Netherlands, June 28-July 3, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00276-08 LCS

PERIOD COVERED

~~October 1, 1985 through September 30, 1986~~

(80 characters or less. Title must fit on one line between the borders.)

Metabolism of Melatonin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH

COOPERATING UNITS (if any)

Section on Neuroendocrinology, LDN, NICHD Department of Pediatrics, USUHS
Office of the Chief, LCS, NIMH Clin. Psychobiol. Branch; NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was inactivated early in the year and subsequently terminated.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00277-08 LCS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Synthesis of Stable Isotope-Labeled Compounds

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH
 Others: Adrian Weisz, Visiting Fellow, LCS, NIMH
 Riccardo Boni, Visiting Fellow, LCS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.2

OTHER:

.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Stable- and some radioisotope-labeled compounds have been synthesized to support other laboratory projects. Structural analogues of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were prepared; oxygen-18-labelled-quinolinic acid prepared by exchange for use as an internal standard; synthetic routes for labelled tryptophan and its metabolites are under study.

Objectives:

The synthesis of labeled compounds is an integral program component in the investigation of metabolism and distribution of endogenous and xenobiotic compounds. Other projects in the SAB require labeled or structural analogues in order to trace metabolic pathways, determine kinetics, or accurately measure trace quantities with an internal mass standard.

Methods Employed:

Conventional routes of chemical synthesis employing isotopes and general chemicals have been used. Sufficient 1-methyl-4-(4-amino)phenyl-1,2,3,6-tetrahydropyridine (4'-amino-MPTP) was synthesized (8 gm) to continue both dog and mouse testing of its properties (Z01-MH-00279-05 LCS). The synthetic route used was nitration of 4-phenylpyridine and separation of the 2', 3', 4' isomers by repeated fractional crystallization. The 4'-isomer was reduced with SnCl_2/HCl to the 4'-amino compound; acetylated with acetic anhydride; 1-methylated with iodomethane; hydrolyzed to remove the acetyl group; and then reduced with sodium borohydride to 4'-amino-MPTP. Extensive purification was effected at several stages to produce product with less than 0.1% impurities, in contrast to a shorter synthetic route using a one-step Pd/C-borohydride reduction of methylated nito compound.

Quinolinic acid- $^{18}\text{O}_4$ was prepared by acid catalyzed exchange of quinolinic acid in $\text{H}_2^{18}\text{O}_4$ and was found to be a suitable internal standard for a negative ionization mass spectrometric assay when esterified under non-acidic conditions (Z01-MH-02384-01 LCS)

Major Findings:

The use of each of these synthetic products is described in other annual reports (Z01 MH 00279-05 LCS, Z01-MH-02384-01 LCS).

Significance to Biomedical Research:

The availability of labeled compounds is frequently the limiting step in metabolism projects. A program in analytical biochemistry requires continuing synthetic efforts to prepare stable and radioisotope analogues for the timely and efficient solution to metabolism projects.

Proposed Course:

Additional structural and labelled analogues of MPTP will be prepared for animal studies. Synthetic routes for labelled analogues of tryptophan and its major metabolites kyurenine, anthranilic acid, kynurenic acid, 3-hydroxykynurenine, and 3-hydroxyanthranilic acid will be investigated.

Publications:

Weisz, A., Markey, S.P., and Ito, Y.: Preparative separation of polar unstable compounds (catecholamines) from a synthetic mixture by high-speed counter-current chromatography. J. Chromatography 383: 132-136, 1986.

^{13}C Weisz, A. and Markey, S.P.: Synthesis of D/L-norepinephrine-(phenyl-U- ^{13}C). J. Lab. Compounds & Radiopharm., in press, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00279-05 LCS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology of Neurotoxins

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I.: Sanford P. Markey, Chief, Section on Analytical Biochemistry
 Others: Jan Johannessen, Senior Staff Fellow, SAB, LCS, NIMH
 Song-cheng Yang, Visiting Scientist, SAB, LCS, NIMH
 Hiroya Ikeda, Guest Worker, SAB, LCS, NIMH
 Mark Duncan, Visiting Fellow, IRP, NINCDS
 Miles Herkenham, Senior Investigator, LNP, NIMH
 Kris Bankiewicz, Visiting Fellow, IRP, NINCDS

COOPERATING UNITS (if any)

Laboratory of Neurophysiology, NIMH
 Office of the Director, IRP, NINCDS

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

6

PROFESSIONAL:

4.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The mechanism of action and the metabolism of the parkinsonian-syndrome inducing neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) has been studied in mouse, dog, and monkey. The metabolism of radiolabelled MPTP has shown that only the pyridinium metabolite, MPP+, is accumulated in monkey brain. Distribution of that metabolite with respect to time (1, 3, or 10 days) indicated that MPP+ persists in the striatum and does not migrate toward or co-exist with the dense neuromelanin deposits in the substantia nigral cells. The conditions for the safe handling of animals treated with MPTP was evaluated; MPTP and its metabolites are not vapor borne, and treated animals can be handled safely with normal laboratory protective clothing.

The neurotoxic effects of MPTP in beagle dogs has been evaluated. MPTP produces a profound and permanent loss of cells within the substantia nigra at doses similar to those effective in primates. Dogs exhibit a transient hypokinesia, but regain locomotor capability in spite of the extent of nigral cell loss.

Immunoassay of MPTP and MPP+ has been improved and polyclonal rabbit antibodies characterized for their binding characteristics. Brain extracts contain substances with interfere with antigen-antibody recognition, necessitating chemical separation. A metabolism study of another parkinsonian producing neurotoxin, α -methyl- β -methylaminopropionic acid (BMAA) has been initiated.

Objectives:

The neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in man and monkey result in a parkinsonian syndrome which is nearly indistinguishable from idiopathic Parkinson's disease. By determining the mechanism of MPTP's action in destroying a specific sub-set of dopamine-rich cells in primate brain, rationale therapy to slow or prevent the progressive idiopathic disease process in man might be designed. Further, environmental toxins which may have mechanisms similar to that of MPTP are being investigated. The metabolism of a compound implicated in the high incidence of a parkinsonian syndrome on Guam, α -amino- β -methylaminopropionic acid (BMAA) is being studied to determine if there are characteristic urinary metabolites to indicate dietary exposure.

Methods Employed:

MPTP toxicity is being studied by: qualitative and quantitative observation of animal behavior and locomotion; neurochemical determination of catecholamines and their metabolites in specific brain regions by high pressure liquid chromatography with electrochemical detection (HPLC-EC); determination of the pattern of MPTP₃ distribution, metabolism, and excretion, using radio and stable-labelled MPTP (³H, ¹⁴C, ²H) in mouse and monkey; autoradiography of tissue exposed to labelled-MPTP (Z01 MH 01090-11); and mass spectrometry of isolated metabolites. Enzyme-linked immunoassay and radioimmunoassay procedures are being used to detect MPTP, MPP⁺, and structurally related compounds.

Major Findings:

Studies on the metabolism of ¹⁴C₆-MPTP in mice and monkeys revealed the following:

- (1) Metabolism of MPTP by monkey produces MPP⁺ as the only metabolite detectable after one day. Greater than 98% of the radioactivity persisting in monkey brain after 24 hrs is MPP⁺. Thus, previous observations of other metabolites were due to label exchange and were artifacts of the experimental procedure.
- (2) In the monkey, MPP⁺ is accumulated and stored in intact striatal axons, and not in nigral cell bodies. This pattern of distribution after 24 hrs is accentuated after 3 and 10 days survival. There is no retrograde transfer of MPP⁺ from the axons to the cell bodies which are rich in neuromelanin. Thus, it is unlikely that neuromelanin participates in the neurotoxic action of MPTP, but is probably an indicator of susceptible cells with limited oxidation capacity.
- (3) The 2% of labelled MPTP metabolites which are bound to precipitable macromolecules in monkey brain are probably not relevant to MPTP neurotoxicity. This interpretation is based upon mouse metabolism studies where the effects of agents which block MPTP neurotoxicity (MAO-B inhibitors, dopamine uptake inhibitors) were found to markedly increase the amount of bound metabolite. The inverse correlation between the amount of bound metabolites and neurotoxicity is strong evidence that only MPP⁺ is relevant to neurotoxicity.

(4) Substantia nigra neurons die by slow retrograde degeneration, a fact which may be particularly relevant to idiopathic Parkinson's disease pathogenesis as a striatal rather than nigral disease process.

(5) Procedures for the safe laboratory use of MPTP in animal studies have been developed. This has been a major laboratory safety issue at NIH and many institutions which utilize MPTP as a pharmacological tool. The distribution of ^{14}C -MPTP administered to mice or monkey in environmental chambers permitted the observations that MPTP is not vapor borne after i.v. administration, and that unmetabolized MPTP on excreta or animal bedding may be safely contained and destroyed.

Other studies have concentrated on determining the best conditions for immunoassay of MPTP and MPP^+ using the rabbit antibodies raised to amino-analogues of MPTP and MPP^+ cross-linked to bovine serum albumin. ELISA assay based upon horseradish peroxidase linked to goat anti-rabbit antibody has been the subject of intensive methodological investigation. The assay works well to detect MPTP and MPP^+ -like compounds, and many structural isomers have been analyzed. However, aqueous or ethanol extracts of brain contain factors which inhibit antigen-antibody recognition, and significantly confound the assay sensitivity. Human brain tissue received from tissue banks was surveyed to determine if extracts of Parkinsonian brains contain MPTP or MPP^+ -like factors. However, these studies were preliminary and not definitive due to the lack of assay specificity. In order to correct these problems, radioimmunoassay rather than colorimetric ELISA has been investigated. Results indicate that RIA is both more sensitive and quantitative. Based upon characterization of various rabbit antibody sera using RIA, those sera with highest titer and specificity have been used to test whether an enzyme amplified ELISA might provide the required sensitivity enhancement to preclude non-specific interferences from tissue constituents. Preliminary data suggest that enzyme amplification works to detect antibody at higher dilutions than possible with RIA or conventional ELISA. Translating that sensitivity enhancement to greater sensitivity toward MPP^+ -like antigens has remained problematic.

The neurotoxicity of MPTP in primates and mice has been extensively described. However, the effects of MPTP in other susceptible species has not been well characterized. Previous work had demonstrated that beagle dogs were susceptible to MPTP at doses similar to those effective in primates. These observations have been extended in a recently completed study. Adult beagle dogs of both sexes were injected with MPTP either alone or after pretreatment with a monoamine oxidase inhibitor (pargyline). Groups were sacrificed 2 h, 3 weeks, or 3 months after injection in order to determine acute and chronic effects of MPTP on biogenic amines and their synthetic and degradative enzymes in various brain regions. MPTP caused a massive and permanent loss of striatal dopamine and the loss of cells within the substantia nigra pars compacta. Despite a permanent loss of the nigrostriatal system, the dogs exhibited only a transient hypokinesia lasting one to two weeks. This has important implications for preclinical neurotoxicity testing of drug safety, and suggests reevaluation of the practice of using behavioural indices of neurotoxicity in the dog and applying the findings to man. Further, the tremendous discrepancy in the behavioral consequences of a loss of the nigrostriatal dopamine system between the dog and primate forces a reexamination of the role of this system in sub-primates. Pargyline pretreatment prevented the loss of striatal dopamine and cells from the substantia nigra, but did not prevent a substantial

but reversible decrease in the concentration of dopamine metabolites. This apparent inhibition of monoamine oxidase is due not to suicide inactivation of the enzyme by MPTP, but to reversible inhibition by the pyridinium metabolite MPP^+ selectively in dopaminergic terminals.

The question of environmental factors which may be implicated as causative agents of Parkinson's disease has drawn attention to α -amino- β -methylamino-propionic acid (BMAA) as a neurotoxic constituent of the seeds of *Cycas circinalis*, a plant used for food on the island of Guam. In former times when natives on Guam used cycad flour as a basic food, a parkinsonian syndrome was very common. Animal studies have implicated BMAA as the neurotoxic principle, but little is known about its occurrence or metabolism. Studies have been initiated to define whether there are unique urinary BMAA metabolites to mark exposure to this amino acid.

Significance to Biomedical Research:

The MPTP-lesioned primate has been firmly established as a useful animal model of idiopathic Parkinson's disease in man. The mechanism of action of MPTP may be relevant to the human disease process, and attempts to identify neurotoxic environmental agents may lead to preventative measures for a very common disease of aging.

Proposed Course:

Questions regarding the subcellular localization of MPTP are most important in determining whether MPP^+ is working as a mitochondrial toxin. The metabolism of 4'-amino-MPTP will be further characterized and studies of its action in dogs completed. Procedures to separate compounds related to MPP^+ will be used in conjunction with RIA and ELISA procedures to re-examine human brain tissue.

Publications:

Johannessen, J.N., Savitt, J.M., Markey, C.J., Bacon, J.P., Weisz, A., Hanselman, D.S., and Markey, S.P.: The development of amine substituted analogues of MPTP as unique tools for the study of MPTP toxicity and Parkinson's disease. Life Sci. 40: 697-704, 1987.

Markey, S.P., Weisz, A., and Bacon, J.P.: Reduced paraquat does not exhibit MPTP-like neurotoxicity. J. Anal. Toxicol. 10: 257, 1986.

Markey, S.P. and Schmuff, N.R.: The pharmacology of the parkinsonian syndrome producing neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and structurally related compounds. Medicinal Chem. Res. 6: 389-429, 1986.

Kopin, I.J., Burns, R.S., Chiueh, C.C., and Markey, S.P.: MPTP-induced parkinsonian syndrome in humans and animals. Alzheimer's and Other Age Related Disorders: Strategies in Research and Development (Eds. A. Fisher, C. Lachmann, and I. Hamin), Plenum Press, pp. 519-530, 1986.

Project No. Z01 MH 00279-05 LCS

Markey, S.P., Yang, S.-C., Johannessen, J.N., Burns, R.S., Herkenham, M., and Bankiewicz, K.: Mechanisms of MPTP toxicity. Submitted to the 6th International Symposium on Catecholamines, Jerusalem, Israel, June 14-19, 1987.

Kopin, I.J. and Markey, S.P.: MPTP toxicity: Implications for research in Parkinson's disease. Ann. Rev. Neurosci., in press, 1987.

Markey, S.P.: MPTP - A new tool to understand Parkinson's disease. Discussions in Neurosciences (FESN), Vol. 3, No. 4, 1986.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02384-01 LCS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Quinolinic Acid Metabolism: Role in Neuropathology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Melvyn P. Heyes, Visiting Associate, LNP, NIMH

Other: Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH

COOPERATING UNITS (If any)

Laboratory of Neurophysiology

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

0.5

OTHER

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Humans are afflicted with a wide variety of neurodegenerative diseases, especially of the cerebral cortex and striatum, which result in neuropsychiatric disturbances. Key anatomical and neurochemical characteristics of certain neuropathologic conditions can be replicated in experimental animals by local injections of quinolinic acid (QUIN), an endogenous metabolite of tryptophan (TRP). QUIN has proved to be a potent neurotoxin and convulsant and may therefore be involved in human neuropathology. To investigate such a role, we developed a sensitive, accurate and specific assay for QUIN in brain and cerebrospinal fluid (CSF). We found that brain QUIN is increased by systemic TRP and 3-hydroxyanthranilic acid (3-OHAA) loads. Inhibition of TRP metabolism by pyrazinamide (PYR) facilitates increases in QUIN following TRP administration. These findings are consistent with an etiologic role of QUIN in glutamic aciduria type I, a pediatric disorder associated with impaired metabolism of TRP.

Objective:

One mechanism that may cause nerve cell death in human neurodegenerative disorders is the presence of neurotoxins, derived either from the environment or present as intermediates of metabolism. One group of compounds have been identified as potent excitants of neuronal activity, convulsants and neurotoxins. The first of these 'excitotoxins' to be identified include the weakly toxic endogenous amino acid glutamic acid and the potent, exogenous glutamate analog, kainic acid. Recently quinolinic acid (QUIN), an endogenous metabolite of tryptophan (TRP), was shown to be a potent neurotoxin and convulsant. QUIN may be responsible for neural degeneration and may also have importance in convulsant disorders.

An integral part of investigating a possible role for QUIN in neuropathology is the development of a specific, sensitive, and accurate assay for QUIN in brain. Two previous assays for brain QUIN had been described, both gas chromatography/mass spectrometry (GC/MS) methods, but sensitivity was low and required 100-300 mg of brain tissue for analysis.

A more sensitive mode of mass spectrometric analysis is electron capture negative chemical ionization (CI) which both efficiently forms a molecular anion and fragments QUIN to a lesser degree than previous approaches. Consequently, negative CI GC/MS requires smaller samples and offer more specific detection of QUIN in the sample. We used this technique, and prepared [¹⁸O]-QUIN for use as internal standard. Extensive tests showed the [¹⁸O]-QUIN was the most suitable internal standard and substantially increased the accuracy of QUIN measurement.

This technique was used to determine the effects of systemic administration of QUIN precursors: L-tryptophan (L-TRP), L-kynurenine (L-KYN), and 3-hydroxyanthranilic acid (3-OHAA) on brain QUIN content. In addition, the effects of impairing the catabolism of L-TRP through the glutaryl-CoA pathway was investigated by administration of pyrazinamide (PYR). This drug results in the inhibition of 2-amino-3-carboxymuconic semialdehyde dehydrogenase, the first enzyme in the glutaryl-CoA pathway.

Methods:

[¹⁸O]-QUIN was prepared by heating 6 mol of QUIN in 1 ml of [¹⁸O]-water/3 N HCL for 48 hours at 80°C. QUIN was quantitated as the dihexafluoroisopropanol ester (mass 467 daltons).

Samples were analyzed following GC separation using a Finnigan 3200 chemical ionization quadrupole mass filter with Extrel electronics and a Teknivent data system 1050. The carrier gas was methane. The minimum detectable quantity at a signal to noise ratio of 10:1 was 6 fmol, a 1000-fold improvement over previous assays.

Sample collection and preparation,

Studies were done on male Sprague-Dawley rats weighing 300-350 g. Brain parts weighing 10 to 120 mg were sonicated in perchloric acid containing [¹⁸O]-QUIN (60 or 300 pmol/ml). For the standard curve, 1 ml of the 6% PCA containing 60 or 300 pmol of [¹⁸O]-QUIN was added to known quantities of QUIN

(6-1200 pmol in duplicate or triplicate) dissolved in water. All samples were centrifuged at 12,000 g for 15 min at 0°C. Supernatants were neutralized with 10 M potassium hydroxide. Following centrifugation the aqueous layer was applied to the Dowex columns. Columns were washed with 4 ml water and 4 ml 0.1 N formic acid. QUIN and [¹⁸O]-QUIN were eluted with 5 ml of 6.0 N formic acid, collected into screw capped tubes and immediately frozen in solid CO₂. Samples were lyophilized overnight or evaporated using rotary evaporator.

Major Findings:

QUIN was identified in samples from brain and CSF, as shown by the presence of QUIN-specific ions at mass-to-charge ratio (m/z) 467 and 316 eluting at the correct time from the GC column. Importantly, no ion currents at m/z 471 and 320 were observed at the time of QUIN elution, establishing that [¹⁸O]-QUIN can be used as internal standard. Extensive tests established that quantitation of QUIN using [¹⁸O]-QUIN was over 30 times more accurate than previous assays.

In saline-treated rats, cortical QUIN content was similar in the three regions measured. QUIN content was increased to 1107%, 977%, and 1235% of control in frontal, parietal, and occipital cortex, respectively, 2 h after a systemic L-TRP load. In a second group of rats, L-TRP increased QUIN content in frontal and parietal cortex by 252% and 519% respectively. Cortical QUIN content was unchanged by PYR-treatment. However, co-administration of PYR and L-TRP resulted in a 1.88-fold and a 1.80-fold potentiation of the increase in QUIN in frontal and parietal cortex respectively. L-TRP increased QUIN content of parietal cortex, striatum, and thalamus by 1065%, 1150%, and 1467% respectively. QUIN content was also increased following 3-OHAA administration, but to a greater extent than by L-TRP loading. L-KYN administration increased QUIN to a lesser extent than did either L-TRP or 3-OHAA.

Significance to Biomedical Research:

Quinolinic acid has proved to be a potent neurotoxin and convulsant when injected into the central nervous systems of experimental animals. Quinolinic acid is present in brain and clearly may have etiologic importance in human brain dysfunction. A sensitive, specific and accurate assay for brain and CSF QUIN is therefore a prerequisite to investigating such a role. Considerable time and effort were expended at this stage of the project to establish the necessary assay. We have hypothesized that an increase in brain QUIN may cause the neuropathological characteristics of glutaric aciduria. Our observation that PYR, which blocks the first step in the glutaryl-CoA pathway, increases brain QUIN is consistent with this hypothesis. Experimental animals treated with PYR may serve as a model for this inborn error of metabolism.

Proposed Course:

Four linked approaches will be pursued over the coming year.

1) Cerebral Metabolism.

Continuation of studies in rodents on the metabolism of QUIN in brain. These will include the effects of diet, circulating amino acid concentrations and the effect of drugs and agents already known to influence L-TRP metabolism.

2) Animal Models of Human Neuropathology.

Both ischemia and hypoglycemia induce neurodegeneration which can be blocked by antagonists of NMDA-type excitatory amino acid receptors, suggesting that activation of these receptors mediate nerve cell damage in these situations. QUIN is a potent agonist of these receptors and mediates at least some of its neurotoxic effects by their activation. Perhaps QUIN is involved in the neuropathology of these conditions. This hypothesis is directly testable using the assay for QUIN we have developed. Studies of PYR will continue, particularly with respect to the effects of diet.

3) QUIN Concentrations in CSF.

Measurement of QUIN in the CSF in humans may open a window into QUIN metabolism in brain. If so, it may be possible to determine ongoing QUIN metabolism in human neuropathological states. To investigate how well QUIN concentrations in CSF reflect brain QUIN levels, rhesus monkeys will be given treatments which have been shown to either increase or decrease QUIN concentrations in brain of rodents, and the concentrations of QUIN in CSF will be determined. Later, postmortem studies will be done to fully characterize the relationship between QUIN in brain and CSF.

4) QUIN Concentrations in Human Neuropathology.

QUIN concentrations will be measured in samples of CSF and brain in patients suffering from and who have died from a variety of neurodegenerative, convulsant, and motor disorders, including AIDS. This result will be pursued in the next fiscal year.

Publications:

Heyes, M.P.: Hypothesis: A role for quinolinic acid in the neuropathology of glutaric aciduria type I. Can. J. Neurol. Sci., in press, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00351-13-LCS

PERIOD COVERED

October 1, 1986 - September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Pharmacology of the Central Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David C. Jimerson, M.D., Chief, Section of Biomedical Psychiatry

COOPERATING UNITS (if any)

SAB, LCS, NIMH; SCBB, LCS, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Biomedical Psychiatry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.3

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Studies in the laboratory focused on the effects of altered feeding patterns, stress, and pharmacologic treatments on the regulation of appetitive behavior, satiety responses and body weight, with particular focus on the influence of central catecholamine and endogenous opiate systems. In a series of studies on the behavioral pharmacology of feeding in laboratory rodents begun during the past year, we showed that naloxone decreased sham feeding of sucrose in a dose dependent pattern similar to that produced by the dopamine antagonist pimozone. Preliminary results of a study neonatal treatment of laboratory rodents with opiate agonists demonstrated that these animals have long-lasting alterations in opiate-mediated feeding behaviors. These preclinical studies may assist in the development of animal models pertinent to the clinical disorders of bulimia and anorexia nervosa.

Other collaborative personnel engaged on the project:

S.P. Markey	Chief, SAB	LCS, NIMH
J.A. Stuckey	Guest Researcher	LCS, NIMH
T.R. Insel	Staff Psychiatrist	LCS, NIMH

Project Description:

Objectives:

The purpose of this study is to develop techniques for the assessment of central and peripheral monoamine function, and to assess the relationship of these measures to activity in other physiological and behavioral systems. Behavioral studies in animals have been initiated to examine the role of central monoamines and other neuromodulators, especially endogenous opiate systems, in preclinical models of eating disorder syndromes.

Methods:

Biochemical methods for assay of endogenous monoamine metabolites in tissues and body fluids include gas chromatography-mass spectrometry (GCMS) and high pressure liquid chromatography. Radioimmunoassay techniques are used for assay of hormone levels regulated by monoamine neurotransmitters in vivo. Effects of pharmacologic treatments and behavioral paradigms on neurotransmitter and neuropeptide receptor activity in laboratory rodents is assessed in vitro using selective radioreceptor binding techniques.

Behavioral studies in rodents include measurement of food and water intake and longitudinal changes in body weight, and ratings of appetitive and satiety behaviors. These measures are recorded in response to acute pharmacological challenge, or as longitudinal changes in response to drug treatments or to alterations in diet or housing conditions.

Major Findings:

Over the past year we have begun studies on preclinical models for altered patterns of appetite and satiety observed in patients with eating disorders. Sham feeding provides one possible model for binge-purge episodes in bulimic patients, since in this paradigm animals do not absorb nutrients from ingested food. Clinical studies on neurotransmitter and neuropeptide function in patients with anorexia nervosa and bulimic disorder suggest that central opiate systems may play a prominent role in the pathophysiology of these syndromes. Thus, anorexic patients at low weight have reduced CSF concentrations of beta-endorphin and related peptides. During the past year, we completed a study with rodents showing that naloxone produced dose-dependent attenuation of sham feeding of sucrose solutions with a similar time course to that produced by the dopamine antagonist naloxone. This finding shows that naloxone-induced satiety is independent of gut absorption of nutrients consumed.

Previous studies have shown that neonatal treatments of rats with opiates

produce changes in opiate receptors that persist into adulthood, with some evidence for changes in behaviors which are thought to be mediated in part by endogenous opiates. In a preliminary study of neonatal treatment of laboratory rats with the opiate agonists morphine and trifluodom, we have observed a long term potentiation of naloxone-induced inhibition of feeding behavior in the adult animals. These initial data suggest that factors altering activity of endogenous opiate systems early in life could have residual effects resulting in altered eating behaviors in adulthood.

Significance to Biomedical Research and the Program of the Institute

Investigation of the effects of neurotransmitters, endogenous opiates, and other neuromodulators on feeding behavior may help in the development of animal models for anorexia nervosa and bulimia. This work is important in evaluating the possible role of pharmacological paradigms or specific behavioral experiences, such as chronic stress or chronic dieting, in the development of neurochemical vulnerability to altered eating patterns, and could ultimately help in the development of new pharmacological treatment strategies for patients with eating disorders.

Proposed Course:

Preclinical studies of feeding behavior will further assess the effects of altered dietary patterns, stress, and pharmacologic treatments on natural feeding and on sham feeding, with particular focus on opiate, catecholamine and serotonergic systems. Behavioral results from current studies will be correlated with post-mortem analyses of brain monoamine metabolites, and receptor binding studies involving central opiate and catecholamine pathways.

Publications:

Pohl, R., Ettedgui, E., Bridges, M., Lycaki, H., Jimerson, D.C., Kopin, I.J., Rainey, J.M.: Plasma MHPG levels in lactate and isoproterenol anxiety states. Biol. Psychiatry 22: 1127-1136, 1987.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02289-03-LCS

PERIOD COVERED

October 1, 1986 - September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychobiology of Eating Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David C. Jimerson, M.D., Chief, Section on Biomedical Psychiatry

COOPERATING UNITS (if any)

SCN, LCS, NIMH; SCP, LCS, NIMH; SCN, BPB, NIMH; CNG, NIMH; SCS, NSB, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Biomedical Psychiatry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

3.7

PROFESSIONAL:

2.7

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clinical investigations involving the syndromes of bulimia and anorexia nervosa during the past year included neurotransmitter, neuropeptide, neuroendocrine, metabolic, pharmacologic and related behavioral studies on neurobiologic factors thought to contribute to the etiology of these disorders, and to their variable responsiveness to available treatments. Consistent with preclinical data implicating hypothalamic serotonin dysregulation in impaired post-prandial satiety, administration of the serotonin agonist m-chlorophenylpiperazine (m-CPP) decreased caloric intake in test meal studies. Prolactin responses to m-CPP were significantly blunted in eating disorder patients and in bulimic anorexic patients. Analysis of pharmacokinetic data showed that m-CPP-induced migraine-like headaches in eating disorder patients were related to blood level of the drug, as well as to personal and family history of migraine headache. Analysis of samples from a newly completed lumbar puncture study demonstrated that cerebrospinal fluid levels of homovanillic acid were significantly reduced in patients with severe bulimia, consistent with an alteration in central reward pathways involving dopamine. In preliminary results of a collaborative study of behavioral and neurochemical responses to 2-deoxyglucose, bulimic patients had blunted responses as reflected in hunger ratings and caloric consumption during a test meal. Follow up on our previous findings of dysregulation of opiate and hypothalamic-pituitary-adrenal function in anorexic patients included collaborative studies utilizing the opiate antagonist naloxone and a glucocorticoid antagonist. Interviews continued for the family study of bulimia. Studies on energy metabolism showed that resting metabolic rate was significantly reduced in weight stable bulimic patients, suggesting increased efficiency of energy utilization. Preliminary results indicated a substantial role of the thyroid axis in elevation of resting metabolic rate observed in anorexic patients during weight gain. 241

Other collaborative personnel engaged on the project:

T.D. Brewerton	Medical Staff Fellow	LCS, NIMH
M.D. Lesem	Medical Staff Fellow	LCS, NIMH
H.A. Brandt	Medical Staff Fellow	LCS, NIMH
J.A. Kasset	Clinical Social Worker	LCS, NIMH
E. Obaraznek	Guest Researcher	LCS, NIMH
A. Hegg	Guest Researcher	LCS, NIMH
W.H. Kaye	Associate Professor	Univ. of Pittsburgh
D.L. Murphy	Chief	LCS, NIMH
P.W. Gold	Chief, SCN	BPB, NIMH
W.Z. Potter	Chief, SCP	LCS, NIMH
D. Pickar	Chief, SCS	NSB, NIMH
D. Weinberger	Chief,	CBDB, NIMH
E.S. Gershon	Chief	CNG, NIMH
W.H. Berrettini	Staff Psychiatrist	CNG, NIMH
C. Duncan	Senior Staff Fellow	LPP, NIMH

Project Description:

Objectives:

The purpose of this project is to study the psychobiology of major eating disorders from the standpoint of neurotransmitter metabolism, neuroendocrine and neuropeptide function, cognitive and psychological function, and pharmacologic treatment. The major focus of this project is presently directed toward the syndromes of anorexia nervosa and bulimia.

Methods:

1. Behavioral and psychological assessment: Baseline evaluation of patients and control subjects entails clinical and structured diagnostic interviews; subjective and objective mood, attitude and behavioral ratings; and psychometric approaches. Baseline measures and treatment-related alterations are assessed by research psychiatrists, social worker, psychologist, clinical nutritionist and nursing staff.

2. Neurobiologic assessment: Studies of presynaptic neurotransmitter activity include measurement of the biogenic amines and their major metabolites in blood, urine and cerebrospinal fluid. These studies focus on norepinephrine and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG), dopamine and homovanillic acid (HVA), and serotonin and 5-hydroxyindole acetic acid (5-HIAA). Neurotransmitter receptor activity is assessed through challenge studies using selective pharmacologic probes, with measurement of physiologic, metabolic, neuroendocrine and behavioral responses. Receptor sensitivity is measured in vitro using cellular elements from blood.

Methods used in neuroendocrine protocols are established techniques of measuring baseline hormone levels in blood (cortisol, corticotropin (ACTH), prolactin, growth hormone, triiodothyronine, and thyroid stimulating hormone)

or urine (free cortisol), as well as responses to hypothalamic releasing factors, other pharmacologic probes, and physiologic challenges. These techniques test the integrity of feedback regulation in specific endocrine systems, as well as the sensitivity of neurohormonal and neurotransmitter receptors. Activity of neuropeptide systems in the central nervous system is evaluated by measurement of cerebrospinal fluid peptide concentrations in a baseline state and during treatment.

Nutritional and metabolic state is evaluated using standard biochemical measures in blood and urine, anthropometry, potassium-40 scanning and indirect calorimetry. Cerebral electrophysiologic responses are measured in collaboration with investigators in other branches. Cerebral anatomic and functional imaging studies utilize computed tomography, positron emission tomography, and nuclear magnetic resonance techniques.

Major Findings:

A. Serotonin Function in Relation to Eating Disorder Symptomatology

Preclinical studies in laboratory animals and pharmacologic studies in human subjects indicate that dysregulation of hypothalamic serotonin function can result in impaired post-prandial satiety responses. We have postulated that the bingeing episodes characteristic of bulimia may reflect, at least in part, such dysregulation of hypothalamic serotonin activity. Similarly, this serotonin abnormality is postulated to contribute to bingeing episodes observed in approximately fifty percent of low weight anorexic patients. Moreover, alterations in central nervous system (CNS) serotonin function could play a role in the symptoms of major depression and behavioral impulsivity frequently reported in association with bulimic disorder and anorexia nervosa.

To compare serotonin function in eating disorder patients and healthy controls, we have measured neuroendocrine and behavioral responses to the serotonin receptor agonist m-chlorophenylpiperazine (m-CPP) (in collaboration with Dr. D. Murphy) and to the serotonin precursor L-tryptophan. In results from standardized test meal sessions following drug administration, we have demonstrated that acute administration of m-CPP decreases caloric consumption, similar to results previously reported in laboratory animals. Neuroendocrine results from the completed m-CPP study demonstrated blunted plasma prolactin responses in 26 female bulimic patients at normal weight, in comparison to responses in 15 age-matched healthy volunteers. Prolactin responses following intravenous L-tryptophan administration were significantly blunted only in the subgroup of bulimic patients with major depression. It appeared that patient - control differences in these studies did not result from pharmacokinetic factors, since both subject groups had similar peak post-infusion plasma levels of L-tryptophan (assayed in the Section neuropharmacology laboratory) and of m-CPP (measured in collaboration with Dr. D. Murphy). Neuroendocrine results are also being correlated with such other clinical variables as severity and duration of bulimic symptoms. Initial neuroendocrine results in twelve low weight anorexic patients (ten of whom also had bulimic symptoms) also showed significantly blunted plasma prolactin responses both to m-CPP and to L-tryptophan administration. These

data are consistent with our proposal for a role for altered hypothalamic serotonin function as a predisposing factor toward binge episodes in bulimic patients. In a related study, we completed double-blind controlled therapeutic trials of the serotonin active drug fenfluramine in seven normal weight outpatients with bulimic disorder. Subjects showed decreased frequency of bulimic episodes during drug treatment. Pharmacologic challenge studies in outpatients treated with fenfluramine or with imipramine are being analyzed to evaluate the possible role of increased efficiency of central nervous system serotonin neurotransmission in the therapeutic effects of these drugs in bulimia.

Ratings of somatic symptoms/side effects following administration of m-CPP in a group of 37 eating disorder patients and 15 healthy controls were notable for a significant incidence of migraine-like headaches. Tryptophan infusions did not produce this pattern of headaches. Analysis of the data suggested that the migraine-like headaches were most common in subjects with a history of migraine headaches in first degree relatives. Analysis of pharmacokinetic data showed that headache severity was correlated with peak m-CPP blood level measured during the three hours after drug administration. From the standpoint of migraine, these results indicate that m-CPP may be a valuable pharmacologic probe for extending previous studies linking migrainous symptoms with altered serotonin activity.

B. Central Dopamine Function and Bulimia

While previous neurotransmitter studies in bulimia have focused largely on norepinephrine and serotonin function, the altered eating patterns in bulimia are also consistent with possible involvement of central dopamine systems. Thus, pharmacological studies in laboratory animals have shown that decreased dopamine activity in the lateral hypothalamus leads to hyperphagia and weight gain. Moreover, dopamine appears to be a major link in mesolimbic pathways important in the hedonic aspects of food intake. Increased responsiveness in this pathway could accentuate the reward properties of eating, possibly consistent with a pattern of alternate phobic avoidance of food, and periodic over indulgence in a binge meal. Conversely, diminished responsiveness in this pathway could lead to episodic consumption of large meals as a person attempted to achieve the usual satisfaction expected from a meal. Dysregulation of mesolimbic dopamine could also contribute to the depressive symptoms common in bulimic and anorexic patients.

Measurement of levels of the major dopamine metabolite HVA in CSF provides the most direct index of the rate of presynaptic dopamine release available for clinical studies. Results from our recently completed CSF studies showed that eight severely symptomatic bulimic patients, who reported a history of bingeing at least twice daily, had significantly lower levels of HVA than did the thirteen less severely symptomatic patients or the eleven healthy controls. Although the patients weighed less than the healthy controls, these results appear not to be related to body weight per se, since body weight was not different for the patient groups with high and low binge frequencies. When patients were restudied after three weeks of abstinence from bingeing and vomiting during the hospital treatment program, CSF HVA

values for the severely symptomatic group had increased significantly toward control values.

These results provide preliminary evidence for altered turnover of central dopamine in patients with severe symptoms of bulimia. Follow-up studies should include measures of dopamine receptor activity, along with metabolite measures. It is presently unclear which factors related to the symptom patterns of bulimia, such as recent fluctuations in body weight or chronic alterations in dietary composition, could result in relatively persistent changes in central neurotransmitter activity. Preclinical studies in laboratory animals have shown that acute changes in eating pattern may in fact affect dopamine turnover in hypothalamic and mesolimbic pathways. Additional analysis of the current data is necessary to compare other clinical variables such as depression, family psychiatric history, and menstrual phase at the time of lumbar puncture for the two patient subgroups.

C. Pharmacological Probes of Appetite Regulation in Bulimia and Anorexia Nervosa.

Preclinical studies and pharmacologic investigations in human subjects indicate that endogenous opiates play an important role in the regulation of feeding behavior, as indicated by decreased meal size resulting from pretreatment with opiate antagonists. We previously demonstrated significantly low levels of CSF beta-endorphin and other pro-opiomelanocortin related peptides in low weight anorexic patients. These observations have prompted us to investigate whether opiate dysregulation, either as a preexisting condition or as a result of persistently abnormal eating patterns, could play a role in the preoccupation with food and binge-eating patterns observed in bulimics and in many anorexic patients. Over the past year we completed collection of additional CSF samples in a new group of anorexic patients and in bulimic patients, to evaluate clinical correlates of the CSF measures. In collaboration with Dr. D. Pickar, we have continued behavioral and neuroendocrine studies with high dose infusion of naloxone in eating disorder patients. In a preliminary group of seven anorexic patients studied following weight restoration, naloxone pretreatment resulted in a paradoxical increase in the amount of food consumed during a test meal, in contrast to effects previously described in volunteers and obese subjects. The anorexic effects of naloxone also appear to be blunted in a group of 15 bulimic patients studied during a phase of abstinence from bingeing and vomiting in the research program. Results of neuroendocrine responses to naloxone infusion in the eating disorder patients are currently being analyzed. The behavioral results obtained to date suggest that modulation of eating behaviors by central opiate pathways may be altered in bulimia and anorexia, and may be pertinent in evaluating other evidence for therapeutic responses to opiate antagonists reported in some severe cases of bulimia and anorexia.

In a related study on the possible therapeutic effects of opiate antagonist treatment in obese patients, we have studied the first eight subjects in a double-blind, placebo-controlled outpatient trial with nalmefene in collaboration with Dr. D. Pickar. Behavioral ratings, dietary information and neuroendocrine responses will be compared for the two week

periods of active drug and placebo treatment. This study will provide new information on the question of possible rapid development of tolerance to the effect of opiate antagonist drugs on eating behavior.

As another probe of appetite regulation in bulimia, we have studied the behavioral and neuroendocrine responses to 2-deoxyglucose in patients and controls (in collaboration with Drs. S. Paul and D. Pickar). Preclinical studies have shown that administration of 2-deoxyglucose results in an intracellular glucopeia, with increased appetite and feeding being prominent behavioral effects. These behavioral effects appear to include an interaction with hypothalamic opiate pathways, since pretreatment with naloxone antagonizes the increased eating (but not the increased hunger) resulting from 2-deoxyglucose administration. In initial results, 13 bulimic patients, in comparison to eleven healthy controls, had blunted responses on hunger ratings following administration of the drug, and attenuated 2-deoxyglucose induced increases in caloric consumption on a test meal. These preliminary results provide the first evidence that bulimic patients may develop dysregulation of glucose sensitive hypothalamic pathways involved in the modulation of appetite and food intake.

D. Studies of the HPA Axis in Anorexia Nervosa.

Collaborative studies with Dr. P. Gold on the regulation of the hypothalamic-pituitary-adrenal (HPA) axis in eating disorder patients have continued over the past year. Previous studies from this collaboration demonstrated blunted ACTH responses to corticotropin releasing hormone (CRH) and elevated levels of CRH in CSF in anorexic patients, suggesting that hyperactivity of the HPA axis in anorexic patients originates in the hypothalamus or more rostral brain regions. Demonstration of elevated CRH in low weight anorexic patients is of particular interest because of the anorectic effects of this peptide when administered to laboratory animals. During the past year, we have completed a collaborative pilot study on the neuroendocrine and behavioral effects of the glucocorticoid antagonist RU-38486 in six anorexic patients studied at low weight and following weight restoration. Data from this study are currently being analyzed. The results may help clarify the site of HPA dysregulation in anorexia nervosa.

E. Regulation of Energy Metabolism Eating Disorder Patients

From a clinical perspective, alterations in metabolic control of energy metabolism and weight regulation could contribute to the difficulties in weight stabilization commonly reported by patients with eating disorders. Patients with the syndrome of bulimia frequently complain of a tendency to gain weight, in spite of apparently modest caloric intake. During this past year, we demonstrated that a group of 15 bulimic patients at normal weight had lower resting metabolic rate (as assessed by indirect calorimetry) than did age and sex matched healthy controls. This finding is an extension of our previous observations that bulimic patients are able to maintain a constant body weight with significantly smaller daily caloric intake than healthy controls, and that anorexic patients with bulimic symptoms require fewer calories to maintain their weight than do non-bulimic anorexics. These

findings are of importance because they provide a physiological explanation for the complaint of many bulimic patients that they find themselves particularly prone to gain weight on a typical diet. These results will be compared to clinical variables (including present deviation from expected body weight, duration and severity of bulimic symptoms) and to activity level in physiological systems (including the sympathetic nervous system and the hypothalamic-pituitary-thyroid axis) involved in the regulation of metabolic rate.

Clinical observations suggest that the caloric requirements for weight gain are unexpectedly large for low weight anorexic patients participating in non-pharmacological, behaviorally oriented weight restoration treatment programs. Over the past year we have extended our series of anorexic patients studied longitudinally, assessing body composition using anthropometric measures and metabolic rate using indirect calorimetry. In comparison to results for healthy controls, data from ten anorexic patients showed that during the weight restoration, refeeding phase of the treatment program, the anorexic patients had significantly increased rates of energy metabolism. This data suggested that anorexic patients differ from other low weight subjects in terms of their inability to maintain efficient utilization of caloric intake during weight restoration. Preliminary results of neuroendocrine assays indicate that substantial activation of the hypothalamic-pituitary-adrenal axis plays a significant role in the non-adaptive metabolic response observed during weight gain in anorexic patients.

E. Family Study of Bulimic Disorder

As in anorexia nervosa, there is substantial evidence implicating a relationship between the syndrome of bulimia and affective illness. Bulimic patients have a high frequency of major and minor depressive episodes, and the limited data available from family history studies indicate an elevated frequency of major affective illness in first degree relatives of bulimic probands. In an effort to replicate results from previous family studies, and to extend them to the more reliable family interview method, we are conducting a family study of bulimia in collaboration with Dr. E. Gershon. Data collection continued actively over the past year, with 28 bulimic probands and 130 first degree relatives having been interviewed to date.

In a related study, we examined the time course of onset of bulimic symptoms in anorexic patients admitted to this program between 1976 and 1987. Between 30 and 50 per cent of anorexic patients typically develop symptoms of bulimia during the course of the disorder. Reports in the literature show that bulimic symptoms generally develop within one and one half years after a patient has started restricting food intake and losing weight. This pattern suggests that a low weight episode of anorexia nervosa might be a major predisposing factor in the development of bulimic symptoms in these patients. In our retrospective study of 74 patients diagnosed by research criteria, we observed a progressive increase in the relative proportion of patients who developed bulimic symptoms prior to the onset of their first low weight anorexic episode. Although preliminary because of the limited sample size,

the present study suggests emergence of a new symptom pattern different from that previously reported in the literature. From a psychobiologic perspective, the present data suggest that bulimic anorexic patients may have predisposition to the onset of bulimia not dependent on the prior expression of a low weight anorexic episode.

Significance to Biomedical Research and the Program of the Institute:

The substantial prevalence of bulimic disorder and anorexia nervosa, particularly in the high risk population of high school and college age women, is a significant public health concern. The studies outlined above reflect a targeted focus on neurobiologic systems currently implicated in altered eating patterns and associated psychiatric symptoms observed in these disorders. Further studies on the role of serotonin, dopamine, norepinephrine, endogenous opiates and related neuromodulators may help to identify biological and behavioral vulnerability factors contributing to the onset and persistence of bulimic and anorexic symptoms, and may contribute to the development of new treatment approaches.

Proposed Course:

Along with continued study of behavioral and neuroendocrine responses to pharmacological challenge with serotonin active drugs in anorexic patients, we will be analyzing data from patients and controls to assess the interrelationship within subjects of behavioral, neuroendocrine and CSF metabolite measures. The studies of behavioral and neuroendocrine responses to challenge testing with an opiate antagonist will be completed, as will the collaborative study responses to nalmeferne in obesity. Collaborative studies on HPA axis activity in anorexia will continue with neuroendocrine challenge tests utilizing a glucocorticoid antagonist. Data collection will continue for energy metabolism studies in a comparison group of patients with affective illness and for the family study of bulimia.

Publications:

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Kaye, W.H., Gwirtsman, H.E., Obarzanek, E., George, D.T., Jimerson, D.C., Ebert, M.H.: Caloric intake necessary for weight maintenance in anorexia nervosa: Nonbulimics require greater caloric intake than bulimics. Am. J. Clin. Nutr. 44: 435-443, 1986.

Petersen, R., Kaye, W.H., Gwirtsman, H.E.: Comparison of calculated estimates and laboratory analysis of food offered to hospitalized eating disorder patients. J. Am. Diet. Assoc. 86: 490-492, 1986

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Approach, Ferrari, E. and Brambilla, F. (Eds.), New York, Pergamon Press, 1986, 319-323.

Jimerson, D.C. and Docherty, J.P (Eds), Psychopharmacology Consultation, Washington, D.C., American Psychiatric Press, 1986.

Jimerson, D.C.: Psychopharmacology consultation for bulimia and anorexia nervosa: A clinical overview. In Jimerson, D.C. and Docherty, J.P. (Eds), Psychopharmacology Consultation, Washington, D.C., American Psychiatric Press, 1986, 49-70.

Kaye, W.H., Gwirtsman, H.E., George, D.T., Weiss, S.R., Jimerson, D.C.: Relationship of mood alterations to bingeing behaviour in bulimia. Brit. J. Psychiatry 149: 479-485, 1986.

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Kaye, W.H., Rubinow, D. Gwirtsman, H.E., George, D.T., Jimerson, D.C., Gold, P.W.: CSF somatostatin in anorexia nervosa and bulimia: relationship to the hypothalamic-pituitary-adrenal axis. Psychoneuroendocrinol., in press, 1987.

Kaye, W.H., Gwirtsman, H.E., Brewerton, T.D., George, D.T., Jimerson, D.C. Ebert, M.H.: Serotonin regulation in bulimia. In Psychobiology of Bulimia, Hudson, J.I. and Pope, H.G. (Eds.), Washington, D.C., American Psychiatric Press, in press, 1987.

Jimerson, D.C., George, D.T., Kaye, W.H., Brewerton, T.D., Goldstein, D.S.: Norepinephrine regulation in bulimia. In Psychobiology of Bulimia, Hudson, J.I. and Pope, H.G. (Eds.), Washington, D.C., American Psychiatric Press, in press, 1987.

George, D.T., Brewerton, T.D., Jimerson, D.C.: Comparison of lactate-induced anxiety in bulimic patients and healthy controls. Psychiatry Res., in press, 1987.

Kaye, W.H., Gwirtsman, H.E., George, D.T., Jimerson, D.C., Ebert, M.H.: CSF 5-HIAA concentrations in anorexia nervosa: reduced values in underweight subjects normalize after weight gain. Biol. Psychiatry, in press, 1987.

Kaye, W.H., Berrettini, W.H., Gwirtsman, H.E., Chretien, M., Gold, P.W., George, D.T., Jimerson, D.C., Ebert, M.H.: Reduced cerebrospinal fluid levels of immunoreactive pro-opiomelanocortin related peptides (including beta-endorphin) in anorexia nervosa. Life Sci., in press, 1987.

Kaye, W.H., Gwirtsman, H., Jimerson, D.C., George, D.T., Karoum, F., Ebert, M.H.: Catecholamine function in anorexia nervosa at low weight and after weight restoration. In Proceedings of the Sixth International Catecholamine Symposium, Dahlstrom, A. (Ed.), New York, Alan R. Liss, in press, 1987.

Jimerson, D.C., Kaye, W.H., Lesem, M.D.: Preliminary evidence for low CSF

homovanillic acid in patients with severe symptoms of bulimia. In Proceedings of the Sixth International Catecholamine Symposium, Dahlstrom, A. (Ed.), New York, Alan R. Liss, in press, 1987.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00326-14 LCS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Neuropharmacology and Psychobiology of Depression and Mania

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Dennis L. Murphy, M.D. Chief Section on Clinical Neuropharmacology LCS NIMH

COOPERATING UNITS (if any)

BP, CBP, NIMH; VA Medical Center, Bronx, NY

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This is the final, termination report on this project. No new data have been collected in the past year. The publications listed in this report were discussed in last year's annual report. Our Section's work in the area of the neuropharmacology and psychobiology of depression has been refocused during the past several years on affective symptomatology as it occurs in the context of obsessive-compulsive disorder (see annual report Z01 MH 00336) and in a comparative study of Alzheimer's disease and depression in the elderly (see annual report Z01 MH 00339).

Other collaborative professional personnel engaged on the project:

C.S. Aulakh	Staff Fellow	LCS NIMH
L.J. Siever	Staff Psychiatrist	VA Med Ctr, Bronx NY
E.A. Mueller	Staff Physician	LCS NIMH
T. Sunderland	Staff Physician	LCS NIMH
R.M. Cohen	Section Chief	LCM NIMH
N.A. Garrick	Biologist	LCS NIMH

Project Description:

Objectives: Individuals with depression, mania, and related disorders with affective components, including individuals with depression secondary to other psychiatric disorders (e.g. personality disorders; panic disorder, obsessive-compulsive disorder) and neuropsychiatric disorders (e.g. Parkinson's disease, Alzheimer's dementia), have been studied in attempts to understand the psychological and biological mechanisms involved in therapeutic drug effects in these disorders. As individual differences in psychoactive drug responsiveness appear to depend upon many psychological and biological factors, a variety of study approaches are utilized.

Methods Employed:

Several strategies are currently used to approach an understanding of the mechanism of antidepressant, antimanic and antianxiety drugs in neuropsychiatric patient populations. Baseline, pretreatment biological, psychological and demographic characteristics are assessed to evaluate possible markers of potential patient subgroups or individual patient differences which may be predictive of drug response.

The principal emphasis in our studies over the past several years has involved (a) detailed investigations of the clinical and biochemical effects of monoamine oxidase-inhibiting antidepressants, particularly newer agents with substrate selective actions; (b) the contributions of changes in the brain serotonergic system to the mechanisms of action of psychoactive drugs; and (c) the contributions of interactions between some less-studied neuromodulators and/or neurotransmitters (e.g., the neuropeptides and the trace amines) and the more "classical" neurotransmitters including the catecholamines and serotonin. These approaches have required the development of new *in vitro* assay systems as well as animal model studies, both of which have most frequently been accomplished via joint efforts with collaborators outside the section.

Major Findings:

Because this section's principal clinical efforts both on our 6D inpatient unit and our outpatient space in the past two years have been directed towards two specialized patient populations (Alzheimer's disease patients with neuropsychiatric problems, including depression, and obsessive compulsive disorder) our current findings relevant to this affective disorders project report are mostly from several areas involving data which required

extensive analysis or which was incorporated in reviews, as listed in the publications section of this report. The findings from antidepressant drug investigations in the specialized populations are included in separate project reports (Z01 MH 00336) and (Z01 MH 00339).

Significance to Biomedical Research and the Program of the Institute:

An important cautionary corollary to the intertwined neurochemical consequences of antidepressant drug administration is the recently clarified evidence that antidepressants are therapeutically active in a wide variety of non-affective disorders such as obsessive-compulsive disorder, panic disorder, anxiety disorder, attention deficit disorder, bulimia, enuresis, migraine and the chronic pain syndrome. This therapeutic responsiveness may sometimes be related to improvement in secondary depressive symptoms, but may also clearly occur in the absence of secondary depression. In particular, improvement in the core symptoms of at least some of these disorders (e.g. obsessive-compulsive disorder) may occur without a change in mood.

Many patients with these disorders display psychobiological abnormalities that show many similarities to but also some differences from those observed in patients with affective disorders, despite the frequent absence of affective symptoms. Although an improvement in subclinical or "masked" depression remains one hypothesis linking antidepressant responsiveness to some shared biological abnormalities in this diverse group of diagnostic entities, an alternative hypothesis suggests that patients responding to antidepressants have a core disorder with common psychobiological abnormalities but different clinical and diagnostic presentations. A third hypothesis suggests that the multiple actions of antidepressant drugs (e.g. on adrenoceptors, muscarinic receptors or the serotonin system) may each be differently important in the therapeutic outcome in depressed or other patients with specific or predominant dysfunctions responsive to alterations in one or another of these transmitter systems. An examination of both the similarities and the differences among the affective and non-affective antidepressant-responsive disorders may provide further clues about the mode of action of antidepressant agents across the spectrum of psychiatric disorders, and possibly about psychobiological elements in depression and the other disorders responsive to antidepressant treatment.

Proposed Course:

As indicated above, over the past several years our Section began to investigate the affective features of obsessive-compulsive disorder and of Alzheimer's disease. We gradually began to focus our efforts on other aspects of these disorders as well, as reviewed in other annual reports from our section, and hence we are terminating this project.

Publications:

Murphy, D.L.: Serotonin neurochemistry: A commentary on some of its quandaries. Neurochem. Int. 8: 161-163, 1986.

Murphy, D.L., Aulakh, C.S., and Garrick, N.A.: How antidepressants work: cautionary conclusions based on clinical and laboratory studies of the longer-term consequences of antidepressant drug treatment. In Antidepressants and Receptor Function, from CIBA Foundation Symposium 123. Sussex, UK, John Wiley and Sons, Ltd., 1986, pp. 106-125.

Siever, L.J., Uhde, T.W., Jimerson, D.C., Lake, C.R., Kopin, I.J., and Murphy, D.L.: Indices of noradrenergic output in depression. Psychiatry Res. 19: 59-73, 1986.

Murphy, D.L., Sunderland, T., Garrick, N.A., Aulakh, C.S., and Cohen, R.M.: Selective amine oxidase inhibitors: basic to clinical studies and back. In Dahl, S.G., Gram, L.F., Paul, S.M., and Potter, W.Z. (eds.): Clinical Pharmacology in Psychiatry, Series III. Selectivity in Psychotropic Drug Action - Promises or Problems? Heidelberg, FRG, Springer-Verlag, 1987, pp. 135-146.

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Murphy, D.L., Cohen, R.M., Sunderland, T., and Mueller, E.A.: Selective monoamine oxidase inhibitors in treatment-resistant depression. In Zohar, J. and Belmaker, R.H. (eds.): Special Treatment for Resistant Depression. New York, PMA Publishing Corp., 1987, pp. 257-268.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00332-09 LCS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Animal Models for the Study of Neuropharmacologic Effects

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Charanjit S. Aulakh

Staff Fellow, LCS, NIMH

COOPERATING UNITS (if any)

LCM, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Neuropharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.6

PROFESSIONAL:

1.3

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

Intravenous administration of the 5-HT_{1B} receptor agonist m-chlorophenylpiperazine (m-CPP) to rats produced increases in plasma prolactin and corticosterone concentration and a decrease in plasma growth hormone. Short-term (2-4 days) or long-term (3 weeks) treatment with either the tricyclic antidepressant imipramine or the monoamine oxidase (MAO) type A inhibiting antidepressant clorgyline did not have any significant effect on the baseline levels of either of these hormones. However, long-term but not short-term imipramine treatment potentiated m-CPP's effect on plasma prolactin, while long-term but not short-term clorgyline treatment attenuated m-CPP's prolactin effect. Neither antidepressant altered m-CPP's effects on corticosterone or growth hormone. These results indicate that various agents effective in different types of affective disorders exert differential modulatory influences on serotonergic mechanisms mediating neuroendocrine responses in vivo.

Other collaborative professional personnel engaged on the project:

D.L. Murphy	Chief,	LCS, NIMH
R.M. Cohen	Section Chief,	LCS, NIMH
J. Zohar	Visiting Associate	LCS, NIMH
K.M. Wozniak	Visiting Associate	LCS, NIMH
G. Bagdy	Visiting Fellow	LCS, NIMH
J.L. Hill	Biostatistician	LCS, NIMH

Project Description:

The serotonergic neurotransmitter system is considered to play a pivotal role in the etiology of a number of neuropsychiatric disorders. In recent years, radioligand studies have demonstrated the existence of various subtypes of 5-HT receptors. We have conducted a series of experiments to correlate these various 5-HT receptors subtypes with different behavioral, neuroendocrine and other physiologic functions. Furthermore, we have used selective 5-HT subtype agonists as challenge agents to explore functional adaptational changes in the serotonergic neurotransmitter system following the long-term administration of antidepressant drugs. These adaptive changes might help in understanding the molecular mechanisms responsible for both the therapeutic and side effects of these drugs.

Methods Employed:

Under halothane anesthesia, the left femoral artery and vein were cannulated in each animal and the catheters were exteriorized subcutaneously at the back of the neck. Saline or various doses of m-CPP were injected intravenously at least 48 hours after the surgery. In the antidepressant studies, clorgyline (1mg/kg/day) or imipramine (5mg/kg/day) or saline was subcutaneously administered continuously by means of osmotic minipumps for 28 days. Blood samples were drawn from the femoral artery and collected in heparinized tubes. Following centrifugation, plasma samples were collected. The plasma concentrations of prolactin, corticosterone and growth hormone were measured by radioimmunoassay.

Major Findings:

Intravenous administration of the 5-HT_{1B} receptor agonist m-chlorophenylpiperazine (m-CPP) to rats produced increases in plasma prolactin (peak effect at 15 minutes), corticosterone (peak effect at 30 minutes) and a decrease in plasma growth hormone (peak effect at 15 minutes) concentrations. Administration of m-CPP produced dose-related changes only in prolactin levels but not in corticosterone or growth hormone levels. Long-term but not short-term treatment with the tricyclic antidepressant imipramine potentiated m-CPP's effect on prolactin but not on corticosterone or growth hormone. These findings suggest the development of functional supersensitivity of 5-HT_{1B} receptors involved in prolactin secretion following long-term imipramine treatment.

In another study, long-term but not short-term treatment with the MAO-type A inhibiting antidepressant clorgyline attenuated m-CPP's effect on prolactin but not on corticosterone or growth hormone. These findings suggest the development of functional subsensitivity of 5-HT_{1B} receptors involved in prolactin secretion following long-term clorgyline treatment. The demonstration of dose-related changes in prolactin but not in corticosterone or growth hormone following m-CPP administration on the one hand and potentiation or attenuation of m-CPP's effect on prolactin but not corticosterone or growth hormone following long-term imipramine or clorgyline treatment respectively, on the other hand suggests either a differential regulation of these hormones by serotonergic mechanisms or different 5-HT receptors subtypes mediating different neuroendocrine functions.

Significance to Biomedical Research and Programs of the Institute:

Since the serotonergic neurotransmitter system is considered to play a pivotal role in the etiology of the affective illness, the demonstration of functional adaptational changes in this neurotransmitter mechanism following long-term antidepressant drug treatment are important. The net sensitivity changes of m-CPP following long-term antidepressant drug treatment observed in the present study are of particular interest since m-CPP is a metabolite of the antidepressant, trazodone and, therefore, may contribute to the pharmacologic and therapeutic effects of trazodone. The present as well as our previous findings with m-CPP in various animal models help validate the use of m-CPP as an index of central serotonergic function in investigations in humans. The present findings also indicate that 5-HT agonist-induced changes in prolactin levels may be a better neuroendocrine measure for assessing serotonergic function following long-term antidepressant drug treatment.

Proposed Course:

Several papers describing these findings have been submitted for publication. During the next year, we will continue to explore functional adaptational changes in the serotonergic system using other 5-HT subtype agonists as challenge agents as well as other animal behavioral models. In a separate series of experiments with fawn-hooded rats, a rat strain with a peripheral platelet storage pool disease, we will examine whether this rat strain also possesses altered central serotonergic function.

Publications

Aulakh, C.S., Cohen, R.M., Hill, J.L., Murphy, D.L. and Zohar, J: Long-term imipramine treatment enhances the locomotor and food intake suppressant effects of m-chlorophenylpiperazine in rats. Br. J. Pharmacol., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00336-08 LCS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Phenomenology and Treatment of Obsessive-Compulsive Disorder in Adults

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T. R. Insel	Staff Physician	LCS NIMH
Others:	D. L. Murphy	Chief	LCS NIMH
	J. Zohar	Visiting Associate	LCS NIMH
	R. Zohar-Kadouch	Visiting Fellow	LCS NIMH
	C. Benkelfat	Visiting Fellow	LCS NIMH
	M. Pato	Medical Staff Fellow	LCE NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science, NIMH

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.7

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Obsessive-compulsive disorder has been studied from several different perspectives since this project began in 1980. During the past year, we have focused primarily on the treatment of this disorder. Previously we showed that the tricyclic antidepressant clomipramine was specifically anti-obsessional, in contrast to several other antidepressants. As clomipramine is considerably more potent than other tricyclic antidepressants in its serotonergic effects, we hypothesized that these effects might be important for its anti-obsessional properties. To test this hypothesis, we extended our earlier findings that the selective serotonin postsynaptic receptor agonist m-chlorophenylpiperazine (m-CPP) increased obsessional symptoms by retesting obsessive-compulsive disorder patients after chronic treatment with clomipramine. Following treatment, patients showed less of a response to m-CPP, suggesting that clomipramine was associated with postsynaptic subsensitivity. Furthermore, metergoline, a serotonin receptor antagonist, appeared to partly reverse the effects of chronic clomipramine treatment. These results, taken together with recent findings from other investigators correlating clinical improvement on clomipramine with changes in serotonin function, strongly suggest that the drug's serotonergic effects are integral to its clinical efficacy for obsessive-compulsive disorder.

Although we have learned much about the biochemical mechanism of clomipramine's action, results from another study reminded us of the limitations of this treatment. Of 18 patients discontinued from chronic clomipramine treatment, 17 relapsed within 7 weeks. Clearly, the drug is a treatment not a cure. Our continued follow-up studies of these patients have revealed the importance of nonpharmacologic factors in outcome.

Objectives: In this, the eighth year of this project investigating the psychobiology of obsessive-compulsive disorder, we addressed two major questions.

Question 1: Are clomipramine's anti-obsessional effects mediated by its actions on serotonergic receptors? We knew from previous studies that (a) clomipramine was more potent than other tricyclic antidepressants for the relief of the symptoms of obsessive-compulsive disorder and (b) clomipramine was more potent than other tricyclic antidepressants for the inhibition of serotonin reuptake. By defining a pharmacologic mechanism for anti-obsessional effects, new compounds might be developed for the treatment of this complex illness.

Question 2: How long do obsessive-compulsive disorder patients need to remain on clomipramine? As this disorder is considered chronic, it is important to determine whether treatment should continue for 6-12 months, as with the treatment of depression, or for extended maintenance as with the treatment of schizophrenia.

Methods Employed: Treatment studies are conducted in the NIMH Outpatient Clinic at the Clinical Center. Local patients with obsessive-compulsive disorder are accepted if they have been ill for at least 1 year, are willing to stop all psychotropic medication, and do not require hospitalization. Clomipramine is given orally in doses up to 300 mg/day. To test for changes in response to a serotonergic challenge, the serotonin postsynaptic agonist, m-chlorophenylpiperazine (m-CPP), is given orally in a single dose (0.5 mg/kg) in a double-blind, placebo controlled trial prior to and at least 8 weeks following the onset of clomipramine treatment. Behavioral measures, including self and observer ratings, are completed following m-CPP and placebo trials--both before and after clomipramine treatment. In addition, blood is collected via an in-dwelling cannula to assess changes in plasma prolactin and cortisol.

In a separate study, patients receive the serotonin receptor antagonist metergoline following chronic clomipramine administration. Metergoline (4.0 mg) is given orally for 4 consecutive days, again under double-blind placebo-controlled conditions. Behavioral and endocrine changes are monitored through both the 4 days of metergoline and 4 days of placebo administration--with the two treatments at least 2 weeks apart. Clomipramine is taken daily throughout the study.

To determine the appropriate length of treatment, 18 obsessional patients who have been treated with clomipramine between 6 and 36 months are being switched from their medication to placebo under double-blind conditions. Ratings of behavior and mood are collected for 6 weeks prior to the switch and for 7 weeks subsequently.

Finally, we are continuing our follow-up studies of previously treated patients including face-to-face interviews, administration of the SADS-anxiety structured interview, self and observer ratings, and the MMPI.

Major Findings:

Clomipramine and serotonin--m-CPP. Although previous studies with m-CPP revealed minimal behavioral effects in healthy volunteers, we noted highly significant increases in ratings of obsessions and anxiety in 12 obsessional patients prior to clomipramine treatment. Endocrine (i.e. prolactin and cortisol) responses to m-CPP were not different between untreated patients and controls. Following chronic clomipramine treatment, the nine patients who were rechallenged with m-CPP did not show significant increases in ratings of obsessions. This hypo-responsiveness to m-CPP was not correlated with clinical improvement. Endocrine responses to m-CPP were not significantly different on clomipramine, although baseline plasma prolactin was nearly doubled from its pretreatment value.

Clomipramine and serotonin--metergoline. In our previous studies, we determined that metergoline did not increase obsessive-compulsive symptoms when given as a single dose. Following chronic clomipramine treatment however, 10 patients showed an overall slight but significant increase in obsessive-compulsive symptoms during the metergoline trial compared to placebo. Metergoline administration was also associated with a decrease in prolactin in the patients on chronic clomipramine treatment.

Length of treatment. Discontinuation of clomipramine was associated with relapse in 17 of 18 obsessive-compulsive patients, even after 2-3 years of treatment. Increases in depression and obsessions were observed within 7 weeks of discontinuation. Length of treatment was not related to relapse.

Curiously, follow-up of 20 patients from our 1980-1983 cohort revealed only one on clomipramine. In general, these ex-patients continued to have significant obsessive-compulsive symptoms, but in most cases the symptoms were not disabling. As an exception, one patient from the original cohort suicided due to a persistent obsessional symptom. In the remainder of the group, improvement was associated with work, interpersonal relationships, and higher levels of premorbid functioning.

Significance to Biomedical Research and the Program of the Institute: With the recent finding that obsessive-compulsive disorder is 40-60 times more common than previously reported--with a higher prevalence than schizophrenia, panic disorder, and anorexia nervosa--the importance of a safe and effective treatment has been recognized. Our research program since 1980 has demonstrated the effectiveness of clomipramine (1980-1982), the relative ineffectiveness of structurally related compounds (1983-1984), and the role of serotonin in the mediation of these effects (1985-1987). Results from this year's research not only describe a potential mechanism for the drug's anti-obsessional effects, they reveal the practical difficulty of relapse when the drug is stopped and the importance of nonpharmacologic factors in long-term outcome.

Proposed Course. In the coming year, we will be returning to our earlier focus on the psychopathology of this disorder. Specifically, our earlier studies (1986) of cortical blood flow in obsessional patients will soon be completed.

We hope to extend this study to the ^{15}O technique to allow an analysis of the role of subcortical structures, such as the striatum, in the pathophysiology of obsessions and anxiety. In addition, we plan to use the PET scan to visualize ^{18}F -2DG uptake in this same region. Ultimately, by combining these novel imaging techniques with clomipramine treatment, we hope to better understand both the pathophysiology and the pharmacotherapy of this intriguing disorder.

Publications:

Insel, T.R., and Akiskal, H.: Obsessive-compulsive disorder with psychotic features: A phenomenologic analysis. Amer. J. Psychiatry 143: 1527-1533, 1986.

Insel, T.R., and Zohar, J.: Psychopharmacologic approaches to obsessive-compulsive disorder. In Meltzer, H.Y. (Eds.): ACNP: A Generation of Progress. New York, Raven Press, 1987, pp. 1205-1210.

Zohar, J., and Insel, T.R.: Obsessive-compulsive disorder: Psychobiological approaches to diagnosis, treatment, and pathophysiology [A.E. Bennett Award paper]. Biologic Psychiatry 22:667-687, 1987.

Zohar, J., Foa, E.B., and Insel, T.R.: The treatment of obsessive-compulsive disorder. APA Manual for Psychiatric Treatments (in press).

Zohar, J., and Insel, T.R.: Diagnosis and treatment of obsessive-compulsive disorder. Psychiatric Annals (in press).

Zohar, J., and Insel, T.R.: Psychopharmacologic treatment of OCD. J. Affective Disord. (in press).

Zohar, J., and Insel, T.R.: Biologic approaches to the diagnosis and treatment of obsessive-compulsive disorder. In Risch, S.C. and Janowsky, D. (Ed.): The Art of Psychopharmacology. New York, Guilford Press (in press).

Zohar, J., Insel, T.R., Foa, E.B., Skeketee, G., Berman, K., Weinberger, D., and Cohen, R.M.: Physiological and psychological changes during in vivo exposure and imaginal flooding of obsessive-compulsive disorder patients. Proceedings of 1985 World Congress of Psychiatry (in press).

Zohar, J., Mueller, E.A., Insel, T.R., Zohar-Kadouch, R., and Murphy, D.L.: Serotonin receptor sensitivity in obsessive-compulsive disorder: comparison of patients and healthy controls. Arch. Gen. Psychiatry, in press.

Zohar, J., Insel, T.R., Zohar-Kadouch, R.C., Hill, J.L., and Murphy, D.L.: Serotonergic responsivity in obsessive-compulsive disorder: effects of chronic clomipramine treatment. Arch. Gen. Psychiatry, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00337-08 LCS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropharmacology of Neuroendocrine and Neurotransmitter Regulatory Mechanisms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Dennis L. Murphy, M.D. Chief Section on Clinical Neuropharmacology LCS NIMH

COOPERATING UNITS (if any)

Centre for Reproductive Biology, Edinburgh, Scotland; BP and CP, NIMH; NIB and LNRI, NINCDS

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.1

PROFESSIONAL:

2.0

OTHER:

1.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

m-Chlorophenylpiperazine (m-CPP), a direct serotonin receptor agonist, has been studied extensively this year by our group in rodents, monkeys, and humans. Neuroendocrine, temperature, cardiovascular, and behavioral effects of m-CPP have been characterized. Studies using m-CPP as a probe of serotonin CNS function in different psychiatric patient groups and during psychoactive drug treatment conditions are underway.

A distinct circadian rhythm in corticotropin releasing hormone was found in cerebrospinal fluid in a study in rhesus monkeys. This rhythm was over twelve hours out of phase with that of cortisol in cerebrospinal fluid or plasma, and is hypothesized to reflect non-hypophysiotropic functions of this peptide, which is well-known to be widely distributed in many brain areas outside of the hypothalamic-pituitary system.

Marked effects of antidepressant drugs, especially MAO-inhibitors, have been observed on plasma and cerebrospinal fluid melatonin in monkeys and humans; changes in N-acetylserotonin and serotonin accompanying diurnal melatonin rhythms have also been observed in monkey cerebrospinal fluid.

Antibodies to beta-endorphin, somatostatin and other neuropeptides have been identified and characterized in human plasma.

Other collaborative professional personnel engaged on the project:

G. Bagdy, Ph.D.	Visiting Fellow	LCS NIMH
N.A. Garrick, Ph.D.	Biologist	LCS NIMH
P.W. Gold, M.D.	Section Chief	BP NIMH
J.L. Hill, Ph.D.	Biostatistician	LCS NIMH
S.P. Markey, Ph.D.	Section Chief	LCS NIMH
H.F. McFarland, M.D.	Section Chief	NIB NINCDS
D.E. McFarlin, M.D.	Lab Chief	NIB NINCDS
J.W. Rose, M.D.	Staff Physician	VA Salt Lake City, Utah
B.F. Roy, M.D.	Staff Physician	VA Washington, D.C.
K. Szemerédi, Ph.D.	Visiting Fellow	LNRI NINCDS
L. Tamarkin, Ph.D.	Staff Fellow	CPB NIMH
P. Taylor, Ph.D.	Chemist	Centre for Reproductive Biology, Edinburgh
T.P. Tomai	Chemist	BP NIMH
Z. Zukowska-Grojec, Ph.D.	Guest Researcher	LNRI NINCDS

Project Description:

Objectives: The discovery that many newly-characterized peptides and hormones are present in high concentrations in the brain, cerebrospinal fluid (CSF), and plasma has led to an entire field of inquiry into the interactions among peptides, hormones, and both the classical monoamine neurotransmitters as well as trace amines in brain. All of these substances may function as modulators of neurotransmission. This project has focused on the measurement of various peptides, hormones, and several monoamines and their metabolites in cerebrospinal fluid and plasma in an attempt to evaluate (a) CNS and other physiologic influences on hormones and monoamines; (b) the relationship between peripheral, brain, and CSF peptide levels, and (c) in particular, to assess the effects of drugs (especially agents with selective actions), as well as stress and other stimuli on monoamines, peptides, and hormones using biochemical, behavioral, neuroendocrine, and other physiologic response measures.

Methods Employed:

Human plasma is obtained from blood samples collected via indwelling venous catheters. Cerebrospinal fluid from non-human primates is collected by means of indwelling lumbar or lateral ventricular cannulae for continuous flow through a refrigerated line into a fraction collector housed in a freezer. Plasma from the non-human primates and rodents is obtained by use of indwelling venous catheters which are usually implanted 15 to 24 hours prior to our studies to permit investigations under non-stressful, basal conditions. Some examples of hormones measured by radioimmunoassay include cortisol, prolactin, growth hormone, beta-endorphin, melatonin, ACTH, and vasopressin. Antibodies to beta-endorphin, ACTH, somatostatin and other peptides are determined by enzyme-linked immunosorbent assay (ELISA). Serotonin and N-acetyl-serotonin are measured by capillary mass spectrometry. Monoamines and monoamine metabolites are measured by high performance liquid chromatography with electrochemical detection.

Major Findings:

In studies investigating possible modulatory function of serotonin on neuroendocrine mechanisms, behavior, and other physiologic functions, dose-dependent responses to the serotonin agonist, m-chlorophenylpiperazine (m-CPP), in humans, monkeys, and rodents have been documented. In monkeys, m-CPP induced neuroendocrine and behavioral changes accompanied by alterations in cerebrospinal fluid 5-hydroxyindoleacetic acid concentrations, without changes in other monoamine metabolites, confirming data obtained with serotonin antagonists in all species indicating that m-CPP acts primarily through brain serotonin mechanisms.

In addition to increasing plasma prolactin, ACTH, cortisol and vasopressin, and increasing temperature, m-CPP was found to have unexpected cardiovascular effects. In rats, blood pressure and heart rate were increased in a dose-dependent fashion by m-CPP. These effects were also observed in pithed rats and in adrenal demedullated rats, indicating that m-CPP was acting directly on the peripheral cardiovascular system. Mediation of m-CPP's effects via serotonin receptors was suggested by studies with antagonists, since metergoline (and, in the case of the pressor responses, ritanserin) blocked m-CPP's effects, while noradrenergic and other antagonists were ineffective.

Further studies are needed on m-CPP's neuroendocrine effects, but in initial experiments to evaluate whether corticotropin-releasing hormone (CRH) might be affected by m-CPP, baseline experiments revealed a marked circadian rhythm in CRH in rhesus monkey CSF. Peak CRH values of 80 pg/ml occurred in the evening at 7:30 pm, while the CRH nadir of 30 pg/ml occurred at approximately 8:00 am. This rhythm was inverse to that of CSF and plasma cortisol, which peak in the morning at 8-9 am.

Antibodies to two peptides, beta-endorphin and somatostatin, were identified and characterized for the first time in studies using human plasma. Among the more than fifty subjects studied to date, the concentrations of the immunoglobulin G specific to beta-endorphin were highest in plasma from individuals with major depression.

Significance to Biomedical Research and the Program of the Institute:

Serotonin, melatonin, and related indoleamines participate in the regulation of behavior, sleep, locomotor activity, reproductive function, and influence several different hormones, including ACTH, CRH, cortisol, prolactin, and vasopressin. Abnormalities in these functions are found in depression and some other psychiatric disorders. The neuroendocrine, temperature, and behavioral responses found using the serotonin receptor agonist, m-CPP, further advance our hopes that this agent may be of value as a probe of the status of central serotonin receptors in various psychiatric disorders and during treatment with antidepressant and other drugs thought to act, in part, via serotonergic mechanisms. In fact, evidence obtained this year from patients with depression, bulimia, and obsessive-compulsive disorder suggests that neuroendocrine and/or behavioral responses to m-CPP are different from those in normal controls.

The findings of a marked circadian rhythm in corticotropin releasing hormone (CRH) are of special interest. While hypothalamic CRH is regarded as a major physiologic regulator of pituitary ACTH secretion and, thereby, of the circadian and stress-related release of cortisol from the adrenal gland, CRH and CRH receptors are also widely distributed in other brain areas of primates and rodents. The marked difference in the circadian rhythm of CRH versus cortisol suggests that CRH in CSF reflects or mediates some non-hypophysiotropic brain functions of this peptide.

The studies of melatonin, n-acetyl-serotonin, and serotonin in CSF suggest that MAO-inhibiting antidepressants (especially the MAO-A selective inhibitor, clorgyline) markedly alter pineal gland function via a mechanism different from that of the tricyclic antidepressants, i.e. by increasing the availability of melatonin's indoleamine precursors. This interesting difference is of relevance to theories regarding the mechanism of action of antidepressants in rodent brain, as a reduction in melatonin release during chronic tricyclic treatment in rodents had been suggested to be a reflection of the functional importance of beta-receptor down-regulation. The fact that net output of melatonin is higher during chronic MAO-inhibitor treatment suggests that the beta-receptor down-regulation is superceded by the increased precursor availability in the case of MAO inhibitors. This constitutes a valuable lesson in emphasizing the need to focus on the final net consequences of drugs to properly assess their mechanism of action.

Many peptides function as neuromodulators in brain, and the more complete delineation of their localization will be of help in defining their function. Antibodies to neuropeptides in human plasma have not previously been identified, and further study in larger patient and control populations is needed to evaluate their significance.

Proposed Course:

Based on our studies with m-CPP in rodents, monkeys, and humans, we have begun to use this central serotonin agonist as a probe to evaluate possible abnormalities in serotonin function in various psychiatric disorders. Collaborations employing m-CPP have been established with other groups within NIMH to evaluate patients with eating disorders, panic disorders, and borderline personalities, and in other NIH institutes and elsewhere to evaluate possible antinociceptive and cardiovascular effects of m-CPP. In regard to the neuropeptides, explorations by Dr. Benajmin Roy are underway regarding the occurrence of antibodies to other peptides in human plasma and their relationship to such neuropsychiatric disorders as depression and Alzheimer's disease.

Publications:

Roy, B.F., Rose, J.W., McFarland, H.F., McFarlin, D.E., and Murphy, D.L.: Anti-beta-endorphin immunoglobulin G in humans. Proc. Natl. Acad. Sci. USA 83: 8739-8743, 1986.

Murphy, D.L., Garrick, N.A., Tamarkin, L., Taylor, P.L., and Markey, S.P.: Effects of antidepressants and other psychotropic drugs on melatonin release and pineal gland function. J. Neural Trans. 21: 291-310, 1986.

Murphy, D.L., Garrick, N.A., Hill, J.L., and Tamarkin, L.: Marked enhancement of clorgyline of nocturnal and daytime melatonin release in rhesus monkeys. Psychopharmacology 92: 382-387, 1987.

Bagdy, G., Szemeredi, K., Zukowska-Grojec, Z., Hill, J., and Murphy, D.L.: m-Chlorophenylpiperazine increases blood pressure and heart rate in pithed and conscious rats. Life Sci. 41: 775-782, 1987.

Garrick, N.A., Hill, J.L., Szele, F.G., Tomai, T.P., Gold, P.W., and Murphy, D.L.: Corticotropin releasing hormone: A marked circadian rhythm in primate cerebrospinal fluid peaks in the evening and is inversely related to the cortisol circadian rhythm. Endocrinology, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00339-06 LCS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropharmacology of Cognition and Mood in Geriatric Neuropsychiatry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Trey Sunderland Chief Unit on Geriatric Psychopharmacology LCS NIMH

COOPERATING UNITS (if any)

LCM, NIMH; NIDA; Enzor Research Foundation

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A pharmacologic challenge strategy in Alzheimer patients using cholinergic and non-cholinergic agents has been the focus of this unit for a number of years. We have previously documented that Alzheimer patients are behaviorally and cognitively more sensitive to acute anticholinergic blockade with intravenous scopolamine, suggesting increased functional sensitivity compared to age-matched controls. This year, we have shown that this increased sensitivity is not seen in geriatric depressed patients, another population frequently presenting with cognitive deficits but without documented central cholinergic pathology. This functional sensitivity to anticholinergic agents in dementia patients has potential therapeutic implications, and we are now exploring the sensitivity of this population to a series of cholinergic agonists, including arecoline and nicotine.

Another major thrust of our unit has been the therapeutic usefulness of the selective monoamine oxidase inhibitor (MAOI), L-deprenyl, in dementia patients. Work from this last year has demonstrated that deprenyl has beneficial cognitive and behavioral effects in the Alzheimer population without serious side effects. In addition, study of the biochemical changes which accompany the use of this drug has been helpful in characterizing some of the possible mechanisms of action. While the therapeutic benefit was relatively small compared to the overall devastation of the illness, the positive effects were encouraging, and we are currently in the process of studying the longer-term effects of this drug in demented patients.

Other collaborative professional personnel engaged on the project:

D.L. Murphy, M.D.	Chief	LCS NIMH
H. Weingartner, Ph.D.	Guest Researcher	BPB NIMH
P.N. Tariot, M.D.	Guest Researcher	LCS NIMH
P.A. Newhouse, M.D.	Guest Researcher	LCS NIMH
R.M. Cohen, M.D., Ph.D.	Section Chief	LCM NIMH
M. Gross, M.D.	Staff Psychiatrist	LCM NIMH
E.A. Mueller, M.D.	Guest Researcher	LCS NIMH
A.M. Mellow, M.D., Ph.D.	Staff Psychiatrist	LCS NIMH
B. Lawlor, M.D.	Staff Psychiatrist	LCS NIMH
S. Molchan, M.D.	Staff Psychiatrist	LCS NIMH
J. Grafman, Ph.D.	Staff Psychologist	ETB NINCDS

Project Description:

Objectives:

- (1) To better characterize pharmacologically the cholinergic system in Alzheimer's disease: Deficits in the central cholinergic system remain the strongest therapeutic lead in the study of Alzheimer's disease; yet, replacement cholinergic therapies have not reversed the cognitive impairments associated with the illness. To better understand this apparent paradox, Alzheimer patients and age-matched normal controls are tested in a series of studies with cholinergic agonists and antagonists. Changes in sensitivity to these pharmacologic agents may help further the understanding of residual cholinergic function in Alzheimer's disease and provide leads for more specific therapeutic treatments in the future.
- (2) To investigate biologic markers at baseline and longitudinally in dementia and geriatric depression: Because dementia and depression are the two single biggest problems in geriatric psychiatry and because of the tremendous clinical overlap between these two syndromes, we are investigating the possibility that biologic similarities exist as well. Already, we have discovered several biologic markers which display this overlap, and we are now following these markers under acute treatment conditions and over time to discover if they provide prognostic or therapeutic usefulness.
- (3) To explore new therapeutic modalities in Alzheimer's disease and geriatric depression: An ultimate goal in our investigations is to develop and extend new treatment approaches to these two illnesses. We have already shown that careful administration of monoamine oxidase inhibitors, specifically L-deprenyl, can provide some benefit to Alzheimer patients and are now currently involved in a long-term comparison study of deprenyl. We continue to study the therapeutic usefulness of cholinergic agonists in dementia and will also be expanding those studies to include non-cholinergic agents (i.e., peptidergic: thyrotropin-releasing hormone, TRH, or serotonergic: m-chlorophenylpiperazine, m-CPP).

Methods Employed:

Clinical Assessment: The clinical diagnosis of Alzheimer disease is based on the DSM-III and the NINCDS-ADRDA criteria as well as the Dementia Rating Scale of Hughes and coworkers in St. Louis. The latter scale is an amalgam of multiple scales including the Blessed Dementia Scale, the Face-Hand test, the Pfeiffer short portable mental status questionnaire and others, and provides a measure of severity of illness. The clinical diagnosis is made only after thorough evaluation with the exclusion of any patients suspected of having multi-infarct or other forms of non-Alzheimer dementia. For those patients followed longitudinally until death, the clinical diagnosis will be confirmed by autopsy. The diagnosis of major affective disorder in the elderly is made on the basis of DSM-III criteria.

Behavioral and Psychological Assessment: Mood and other behavioral characteristics are measured with global (15-point) ratings scales, the Brief Psychiatric Rating Scale, and the Hamilton depression rating scale where appropriate. A dementia mood assessment scale (DMAS) has been developed specifically for dementia patients on our unit because of the difficulty encountered with self-rating forms or other mood scales designed for general depressed patients. Activities of daily living are assessed by family and staff throughout the hospitalization with a measurement tool also developed specifically for our geriatric populations as part of this project.

For the evaluation of cognitive skills, a large number of tests are employed. There are several routine psychometric measures including the Wechsler memory quotient in addition to a series of recently designed or modified tasks for this population. These tests assess effortful and semantic memory and include measures of attention, free recall and recognition memory. Though primarily evaluating verbal memory, some of the tests do measure visual memory and sustained motor attention.

Biological Assessment: Plasma, platelets, urine, and cerebrospinal fluid are collected for measurement of enzymes, hormones, levels of biogenic amines and their metabolites. The dexamethasone suppression test and the TRH stimulation tests are also used. Some patients and controls are asked to undergo a skin biopsy for culturing and subsequent biochemical testing of the skin fibroblasts. A major portion of the biologic testing involves pharmacologic challenge studies. Patients and normal volunteers are given intravenous or oral medications (i.e. scopolamine, nicotine, arecoline, TRH, or m-CPP) and followed over the next several hours for physiologic, behavioral, neuroendocrine, or cognitive changes which are then compared to placebo conditions.

Major Findings:

Alzheimer patients have been shown to be more sensitive to central cholinergic blockade than age and sex-matched normal controls along cognitive and behavioral but not physiologic parameters. This increased functional sensitivity was not observed in elderly depressed patients when they were tested over the last year by Dr. Paul Newhouse. In addition, the mostly elderly controls showed evidence, at least briefly, of significant cognitive impairment when given the highest dose of scopolamine (0.5 mg i.v.). By

transiently mimicking the cognitive profile for mild dementia, the scopolamine test therefore provides for a possible pharmacologic modelling of dementia in otherwise normal aged humans.

Continued investigation of the biologic links between depression and dementia has led to the finding of other areas of significant overlap. Cerebrospinal fluid measurement of somatostatin-like-immunoreactivity has been shown to be decreased in both elderly depressives and Alzheimer patients. Following the previously-reported blunted response of thyroid stimulating hormone (TSH) to thyrotropin releasing hormone (TRH) in Alzheimer and elderly depressed patients, we have now also discovered and reported a blunted prolactin response to TRH in both groups compared to normals.

Therapeutically, there has been modest improvement noted in the Alzheimer patients with low but not high doses of L-deprenyl. Ratings of mood and behavior as well as performance on effort-demanding cognitive tasks showed significant change on 10 mg/day of deprenyl (a dose previously shown to be relatively MAO-B selective) over a three-week trial. These behavioral changes were accompanied by slight changes in cerebrospinal fluid (CSF) monoamine metabolites. When the dosage was increased, the CSF metabolites revealed changes more characteristic of a non-selective MAOI, and the behavioral and cognitive improvements were lost. Whether this improvement at low, MAO-B selective doses of deprenyl is specific to Alzheimer patients or might also be seen in elderly depressed patients is currently being investigated.

Significance to Biomedical Research and the Program of the Institute:

Alzheimer's disease still remains a clinical diagnosis of exclusion which is in doubt until autopsy or biopsy confirmation. The ability to establish an antemortem biologic marker for Alzheimer's disease would therefore be of great value for clinicians and researchers alike to establish earlier diagnoses and to reduce diagnostic heterogeneity in studies. While the scopolamine challenge test is not yet such a marker, this pharmacologic probe has revealed significant differences between normals and dementia patients, and now between dementia patients and elderly depressed subjects. This latter comparison is potentially significant clinically because of the frequent overlap between the presenting symptoms of dementia and elderly depression (i.e., pseudodementia). For future investigations, the response to a challenge dose of 0.25 mg of scopolamine might help determine a patient's underlying cholinergic sensitivity and help provide a predictive guide to the individual responsiveness to therapeutic agents, including cholinergic agents such as nicotine or arecoline. In addition, the transient, mild dementia-like picture created by scopolamine in elderly normal controls provides a pharmacologic model of Alzheimer's disease in humans which may serve as a platform for new drug development.

The increasing documentation of biologic links between depression and dementia has already led to significant research and clinical results. By treating Alzheimer patients with an antidepressant such as the monoamine oxidase inhibitor L-deprenyl, we are simultaneously learning more about the underlying biochemistry of the illness and providing at least some benefit to this otherwise unrelenting, progressive illness. The fact that

antidepressants may actually improve some of the symptoms of dementia also opens the door for a whole host of non-cholinergic agents to be tested in what has for years been considered primarily a disease of cholinergic neurons. It may well be that drugs or combinations of drugs that alter neurotransmitter systems other than the cholinergic system have an important role in future treatment strategies.

Proposed Course:

We have already shown that the increased anticholinergic sensitivity in Alzheimer patients is not found in age-matched controls and depressed patients. We must now go on to see if this increased sensitivity is found with other non-cholinergic central nervous system drugs (i.e. benzodiazepines). We must also investigate other demented populations (i.e. Korsakoff's or Parkinson's patients) to see if this sensitivity is specific to Alzheimer's dementia. If the selectivity remains, then exploratory testing with the offspring of demented subjects, in twins, or within families with a high genetic loading of Alzheimer's disease would be valuable. While the ethical considerations of a diagnostic challenge test must be addressed, the potential therapeutic benefit of a more definitive diagnosis and earlier initiation of treatment could be tremendous. We must also test the relationship between anticholinergic sensitivity and response to cholinergic agonists. It is possible, for instance, that previous studies have tested potentially helpful medications too far out on the dose-response curve and have therefore missed the "therapeutic window."

Our study of biologic links between depression and dementia will be enlarged to include family history studies and comparisons of mapping electroencephalograms across diagnostic groups. We will also be attempting to carefully quantify the severity of depression in our demented subjects. Therapeutically, the short-term gains identified in Alzheimer patients with low-dose deprenyl will be compared to other clinically available medications in a longitudinal outpatient study. Acute challenge studies with other potentially therapeutic agents such as TRH, m-CPP, nicotine, and arecoline will also be continued.

Up until now, our projects have been limited to relatively small drug and biologic studies. Over the past five years, however, we have accumulated in depth behavioral, neuropsychological, and biologic data on scores of Alzheimer patients in all stages of the illness. Over the next two years, we plan to combine these expanding datasets to establish a profiling system for Alzheimer's disease. As our project continues and the brain bank inevitably expands, this profiling system will include final pathologic diagnoses. The ultimate biologic fingerprint of baseline and longitudinal information may eventually be quite valuable for both diagnostic and prognostic purposes.

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Newhouse, P.A., Sunderland, T., Tariot, P.M., Mueller, E.A., Murphy, D.L., and Cohen, R.M.: TRH stimulation in Alzheimer's disease (letter). Acta Psychiatr. Scand., in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 MH 00433-07 LCS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Neuropeptides and Biogenic Amines in Neuroendocrine Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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 Others: M. Aiso VF/LCS/NIMH F. Correa Guggenheim Fellow
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 C. Gonzalez VF/NI/NHLBI A.J. Nazarali Alberta Found. Fellow

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

9.0

PROFESSIONAL:

7.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

With the use of quantitative autoradiography, we studied the role of neuropeptides (angiotensin II, atrial natriuretic peptide) and biogenic amines (dopamine) in the central regulation of autonomic nervous system and pituitary gland. We also studied the role of neuropeptides in the regulation of the immune response by localizing neuropeptide receptors in immune organs and immune cells and by investigating the second messenger response.

We demonstrated a role for central angiotensin II and atrial natriuretic peptide in the control of cardiovascular function. These neuropeptides may also be involved in regulation of the immune response. Specific angiotensin II and β -adrenoceptors were quantified in the rat heart conduction system. New methods were developed to study localization and transport of D_1 and D_2 dopamine receptors in brain, and to study localization of malignant melanomas. Psychotomimetic phenylisopropylamine receptors were localized and quantified in selective brain areas.

PROJECT DESCRIPTION

Objectives

To study the role of central and peripheral biogenic amines and neuropeptides in restricted brain areas, and in peripheral tissues involved in cardiovascular function, pituitary control and regulation of immune response. This study includes the continual development of new quantitative autoradiographic methods for determination and quantification of receptors, peptides and related enzymes in rat brain nuclei.

To develop quantitative autoradiographic methods for the study of biogenic amine and neuropeptide receptors in human blood cells, and to apply these methods to clinical studies.

Methods Employed

Neuroanatomical, surgical, biochemical (RIA, gel electrophoresis, radioenzymatic assays) and autoradiography with image analysis combined with computerized microdensitometry.

Major Findings

1. We determined the presence of angiotensin II receptors in a forebrain band, which continues from the subformal organ to the organon vasculosum laminae terminalis and the paraventricular nucleus. This band may represent the link between peripheral and central angiotensin systems.
2. Selective areas corresponding to this forebrain band (nucleus preopticus medianus, organon vasculosum laminae terminalis, and paraventricular nucleus, as well as previously described subformal organ) show higher number of angiotensin II receptors in strains of spontaneously hypertensive rats. Activation of the forebrain band angiotensin system may play a role in the development and maintenance of genetic hypertension.
3. After immobilization stress, rats have a selective increase in the number of angiotensin receptors in the paraventricular nucleus, with no change in anterior pituitary angiotensin receptors. This result suggests that brain angiotensin receptors have a role in the central regulation of the stress response.
4. In the DOCA-salt hypertensive rat, another model of hypertension, an increase in number of angiotensin II receptors occurs in specific brain areas (subformal organ, paraventricular nucleus, nucleus of the solitary tract, median preoptic nucleus), indicating that participation of central angiotensin systems in the control of blood pressure is not restricted to genetically hypertensive animals, and may be a more general phenomenon.
5. Angiotensin II receptors are present in the human adrenal medulla and zona glomerulosa.
6. Receptors for atrial natriuretic peptide were studied in brain and in several peripheral organs of the rat. High receptor concentration was

detected in sympathetic ganglia (superior cervical, stellate and celiac), pituitary gland (anterior and posterior lobes), kidney glomeruli and medulla, adrenal cortex, and immune organs (thymus and spleen), as well as in isolated thymocytes and spleen cells. Incubation of isolated thymocytes and spleen cells with atrial natriuretic peptide results in a concentration-dependent increase in cyclic GMP. Similar results were obtained in isolated sympathetic ganglia.

7. More than an 90% decrease was noted in the number of atrial natriuretic peptide binding sites in sympathetic ganglia, pituitary, thymus and spleen from spontaneously hypertensive rats when compared to that of normotensive control rats. The cyclic GMP increase obtained after incubation of thymocytes and spleen cells with atrial natriuretic peptide, however, was no different in hypertensive than in control rats. This result indicates a possible heterogeneity of the atrial natriuretic peptide binding sites, and suggests that changes in hypertensive rats probably reflect the loss of binding sites not linked to guanylate cyclase.

8. A new method was developed, which allows application of quantitative autoradiography of neuropeptide and biogenic amine receptors to samples prepared from isolated whole cell preparations. This method has wide application to both animal and clinical research, and is ten times more sensitive than classical membrane binding techniques. Adrenergic β -receptors and VIP receptors were quantified with this method in human blood lymphocytes.

9. Several neuropeptide receptors, other than those for atrial natriuretic peptide (including angiotensin II and substance P), were localized in the thymus and spleen of the rat.

10. We developed a dissecting procedure to localize the conduction system of the rat heart. This method is used for quantitative autoradiographic techniques. Specific areas such as the sinoatrial node, atrioventricular node, and intrinsic heart parasympathetic ganglia, can be identified and studied. We localized β_1 and β_2 adrenoceptors and quantified their ratio (about 50/50%) in the sinoatrial and atrioventricular nodes. In addition, we identified angiotensin II receptors in all parts of the rat heart conduction system.

11. The number of both D_1 and D_2 dopamine receptors was quantified in brain areas and in pituitary gland by autoradiography and the use of new, ^{125}I -ligands. We showed that D_1 receptors are present in the caudate-nigral pathway, and demonstrated their bidirectional transport.

12. A specific D_1 dopamine receptor antagonist binds specifically to melanin, and may prove to be a diagnostic agent capable of detecting pigmented melanomas.

13. There is a significant increase, as determined by quantitative autoradiography, in D_1 receptors in the substantia nigra of rats submitted to chronic electroconvulsive treatment.

14. Insulin-like growth factor I (IGF I) receptors were localized in human brain cortex and pituitary gland. The number of these receptors is higher in glioblastomas.

15. As determined by autoradiography, the iodinated 5HT₂ agonist, with psychotomimetic properties, (4-iodo-2,5-dimethoxy-phenylisopropylamine) (¹²⁵I-DOI) binds specifically to rat cortex (layer IV), claustrum, and olfactory tracts. This indicates anatomical selectivity for the psychotomimetic phenylisopropylamines.

16. Specific NPY receptors were located in the zona glomerulosa of the bovine adrenal cortex, suggesting a role for this peptide in control of aldosterone secretion.

Significance to Biomedical Research and to the Institute

Application of quantitative autoradiographic methods to basic research is beginning to yield a significant number of new findings which will help to clarify the role of neuropeptides and biogenic amines in central regulation of cardiovascular function, pituitary control, stress, and immune response. These methods will also help to clarify important anatomical and physiological aspects of the action of psychotomimetic compounds. In addition, quantitative autoradiographic methods may now be applied to clinical studies for the correlation of multiple neuropeptide and amine receptors in human blood samples.

Proposed Course

With the use of quantitative autoradiography, we plan to study further the interactions between neuropeptides and biogenic amines in brain areas involved in the control of pituitary and autonomic function, with emphasis on correlation between binding sites and corresponding second messenger responses. New methods for the quantification of neuropeptides in restricted brain areas will be developed using similar techniques. We will focus these studies on the regulatory mechanisms for cardiovascular, pituitary and immune system function. In addition, we plan to apply our new autoradiographic methods for the study of neuropeptide and biogenic amine receptors in human peripheral blood lymphocytes, under a variety of physiological and pathophysiological conditions, with special emphasis in neuropsychiatric disorders.

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In Press

Castren, E., Kurihara, M., Gutkind, J.S., and Saavedra, J.M.: Atrial natriuretic peptide receptors in thymus and spleen of young spontaneously hypertensive rats. Biologically Active Atrial Peptides, ASH Symposium Series. Vol. II.

Castren, E., Kurihara, M., Gutkind, J.S., and Saavedra, J.M.: Specific angiotensin II binding sites in the rat stellate and superior cervical ganglia. Brain Res.

Castren, E., Kurihara, M., and Saavedra, J.M.: Autoradiographic localization and characterization of angiotensin II binding sites in the spleen of rats and mice. Peptides.

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Saavedra, J.M., Israel, A., Correa, F.M.A., and Kurihara, M.: Increased atrial natriuretic peptide (99-126) binding sites in the subformal organ of water deprived and Brattleboro rats. Proceedings of World Congress on Biologically Active Atrial Peptides.

Saavedra, J.M., Israel, A., Kurihara, M., and Correa, F.M.A.: Decreased number and affinity of rat atrial natriuretic peptide (99-126) binding sites in the subformal organ of spontaneously hypertensive rats. Proceedings of World Congress on Biologically Active Atrial Peptides.

Saavedra, J.M., Kurihara, M., and Israel, A.: Alterations in angiotensin and atrial natriuretic peptide receptors in brain nuclei of spontaneously hypertensive rats. J. Hypertens. (suppl.).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00447-18 LCS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Amine neurotransmitters and metabolites in mental illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

William Z. Potter, M.D., Ph.D., Chief, Section on Clinical Pharmacology,
Laboratory of Clinical Science, NIMH

COOPERATING UNITS (if any)

Clinical Psychobiology Branch; Neuroscience Branch;
Child Psychiatry Branch, NIMH; and Laboratory of Clinical
Studies, NIAAA

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.45

PROFESSIONAL:

2.55

OTHER:

.9

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Alterations of amine neurotransmitter systems (norepinephrine (NE), serotonin (5HT) and dopamine (DA)) have been indirectly implicated in the pathophysiology of the major mental illnesses, depression and schizophrenia. We have applied new techniques to study the interpretation of neurotransmitter measures from cerebrospinal fluid (CSF), plasma and urine from drug-free patients with affective illness and schizophrenia. New findings include the following:

1. The interrelationships between neurotransmitters as evidenced by correlations of metabolites of NE, 5HT and DA in CSF discriminate between responders and non-responders to antidepressant treatment. Moreover, the same measures appear to discriminate drug free schizophrenics from normal controls.

2. The important question of whether variations in at least one type of neurotransmitter receptor on cells obtainable from blood can be explained primarily on the basis of circulating agonist has been answered. Lymphocyte beta receptor parameters have been clearly shown to vary independently of plasma norepinephrine and epinephrine.

3. Longitudinal studies of patients with affective illness have included repeat biochemical measures in drug-free states during which mood was normal. Surprisingly, the exaggerated plasma NE response to going from a supine to standing position which is consistently noted in the depressed state persisted during the "well" state. Thus, investigation of the NE system may reveal a trait abnormality in persons susceptible to affective disorder and provide a means of identifying persons at risk.

Other Professional Personnel:

Matthew Rudorfer	Senior Staff Fellow	LCS/NIMH
John Hsiao	Medical Staff Fellow	LCS/NIMH
Emile Risby	Guest Researcher (NRSA)	LCS/NIMH
Ivan Mefford	Special Expert	LCS/NIMH
Laura Fochtmann	PRAT Fellow	LCS/NIMH
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David Sack	Senior Staff Fellow	CP/NIMH
David Jimerson	Chief, Section on Biomedical Psych.	LCS/NIMH
David Pickar	Chief, Section on Clinical Studies	NSB/NIMH
Judith Rapoport	Chief, Child Psychiatry Branch	CHP/NIMH
Trey Sunderland	Senior Staff Fellow	LCS/NIMH

Project Description:

The characterization of the functional state of three amine neurotransmitter (NT) systems, norepinephrine (NE), serotonin (5HT) and dopamine (DA), in depression and other major psychiatric illnesses such as schizophrenia continues to be a major ongoing project. We have added to that the exploration of ways to characterize the epinephrine (EPI) system. Ongoing method development and clinical studies reveal sources of variance which we can only partly control such as the inherent "stress" responses of NT systems to invasive procedures.

It has become increasingly clear that measures of NT systems covary to a significant degree and may reflect some degree of functional interdependence in the central nervous system. This possibility has led to the reevaluation of NT measures in subgroups of patients and controls in terms of their balance. In the absence of validated models for exploring any "balance" between NT systems non-model dependent techniques such as ratios of two measures or correlation matrices of three or more are employed.

Use of this approach can be applied to the cumulative data base of measures obtained in controls and patients with depression, mania, schizophrenia, eating disorders, attention deficit disorders and Alzheimer's disease from values obtained after 1981 (the data from which new standardized assays were made operational). Both group differences in the untreated state and prediction of response to treatment are explored.

Methods:

Biochemical techniques are described in a separate project summary pertaining to the central laboratory (Z01 MH 01855-03).

Selection of subjects, paying particular attention to such issues as age of onset, frequency of recurrence of episodes, and family history is given great emphasis. Whenever feasible, extended (over 1 month) drug-free periods are required before biological samples are obtained--a 3-

week period is our current minimum although our data show that even this is inadequate in some patients who have been on tricyclic antidepressants and is definitely inadequate in any patient who has received chronic neuroleptics or monoamine oxidase inhibitors. Patients are also characterized according to length on a low monoamine diet as well as number of days in hospital. This latter parameter is of particular interest since many depressed patients are studied after brief (sometimes only overnight) hospitalization and then transferred to outpatient status. With the expansion of outpatient studies, some procedures are performed in some studies without hospitalization.

"Control" subjects must be drawn from both hospitalized and "outpatient" age- and sex-matched individuals who are asked to be on diet. It appears that for comparisons of urine and CSF hospitalization can be a critical variable. Therefore, a comparison of "controls" under different conditions has become an essential component of our design.

Major Findings:

1. Certain interrelationships between neurotransmitters and/or their metabolites continue to emerge as potentially more physiologically relevant than the absolute concentration of substances by themselves. The most robust and consistently observed relationship remains the high correlation between 5HIAA and HVA in the CSF although significant correlations are also observed between MHPG and 5HIAA or HVA in comparison of several hundred pairs of values. For instance, non-responders to drug treatment do not show the usual patterns of correlation between the three NT metabolites in CSF found in controls and responders to treatment. Moreover, in a preliminary analysis of data provided by the 4E schizophrenia research unit, schizophrenics as a group showed a lower degree of correlation than normal controls.

2. In a separate but related series of analyses a previously noted disparity of absolute concentrations of 5HIAA and HVA between control groups has been explored. We find that the ratio of HVA to 5HIAA and the ratio of HVA to MHPG are quite stable across control groups even when the mean values for any single metabolite differ dramatically. And at least in the case of the HVA/5HIAA ratio, it was substantially lower in two separate groups of depressed patients compared to controls and intermediately lower in schizophrenics. In contrast, initial analyses of borderline personality disorder patients reveal a normal HVA/5HIAA ratio. Thus, the ratio provides a new means of identifying biochemical abnormalities in certain patient groups.

3. Attempts to characterize epinephrine in CSF and venous blood using the most sensitive assay available raise questions about the validity of previous claims since resting concentrations are often below the level of detection. In stress paradigms ("learned helplessness," orthostatic challenge and cold exposure of upper extremity) consistent elevations of venous EPI are not observed although other groups have shown robust increases of arterial EPI under comparable circumstances. Thus, alternate approaches to studying EPI function in man are necessary.

4. Sufficient studies have been completed comparing plasma measures of NE and EPI to establish that variations on density or function of lymphocyte beta receptors can not be explained on the basis of circulating catecholamines.

5. Interpretation of plasma concentrations of a metabolite of at least one NT, i.e. HVA from DA, must be reassessed in light of the demonstration that variation in the renal clearance of HVA might account for variations in plasma concentration rather than the rate of production of HVA. Moreover, we have just found that the total excretion of HVA in urine is correlated at the 0.8 or above level with that of both major NE metabolites, VMA and MHPG. Thus, in the periphery it seems unlikely that the bulk of HVA concentration is directly related to DA function in the brain.

6. Expansion of baseline studies from cross-sectional to longitudinal investigations of the course of affective illness has enabled the addressing of state-versus-trait issues. Using plasma and CSF basal measures, bipolar depression cannot be distinguished from drug-free normal mood in the same individuals. Hypomania in these patients is associated with relative increases in resting plasma norepinephrine concentration and in CSF MHPG and HVA concentrations. The most striking finding to emerge is the persistence into euthymia of depression-associated exaggerated reactivity of plasma norepinephrine to an orthostatic challenge. Should this result hold up in further subjects to be studied during the coming year, investigations of noradrenergic reactivity in relatives of depressed patients or other high-risk individuals will be undertaken to assess the potential of this measure as a marker of a depression diathesis.

Significance to Biomedical Research and to the Program of the Institute :

The major theories about the biological causes of the most prevalent severe psychiatric disorders, depression and schizophrenia, center on monoamine neurotransmitter systems. This project applies sophisticated laboratory assays directly to human studies of monoamine metabolism. Results expand our understanding of the role of norepinephrine and other neurotransmitters, mainly in depression. The personal and social costs of this illness are great. Insofar as careful clinical research, drawing on basic biochemical techniques, can identify biological factors in these disorders, specific pharmacologic treatments can be developed and tested in therapeutic trials.

Proposed Course:

We will apply current and develop alternate methods of looking at relationships between neurotransmitter systems to the combined populations of controls and patients made possible by centralization of assays (see separate project No. Z01 MH 01855-02). We believe that this approach provides the best chance of breakthroughs in our ability to use neurotransmitter and their metabolite concentrations as tools in diagnosis, prediction of treatment and understanding pathophysiology. Conversely, we are impressed by the essentially "normal" range of values observed for any single parameter in the resting, drug-free state in a variety of neuro-psychiatric disorders.

A. In light of the emerging group differences in degree of covariance and/or ratios of neurotransmitter metabolites we will focus more on studies of determinants of these composite parameters in control as well as patient populations. Since the first findings related to possible inter-active measures have emerged from studies of CSF we will consider metabolite ratios and covariance as a function of other substances (e.g. peptide hormones) which have previously been reported to correlate with one or another.

B. Alternate approaches to investigating EPI function in man will be pursued in collaboration with groups who are using either insulin or deoxyglucose challenges in patients and volunteers since these metabolic manipulations can produce a centrally-mediated robust release of adrenal EPI as shown in preclinical studies. Urinary measures of EPI and its metabolite metanephrine will also be investigated with the focus on whether they provide information independent of plasma EPI. This will necessarily include measures of the renal clearance of EPI.

C. Our most far-reaching plan is to assess whether the summing of both DA and NE metabolites in urine can provide an index of "whole-body" hydroxylase activity. To this end, free vs total DOPAC must first be considered along with the possible effects of diet on this other major DA metabolite. Pharmacologic manipulations in both humans and animals can then be used to see if decreasing tyrosine hydroxylase activity produces consistent and predictable decreases of the sum of its products in urine. Ultimately this may allow us to establish individual tyrosine hydroxylase "phenotypes" in anticipation of applying molecular genetic studies on this enzyme.

Publications

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Agren, H., Mefford, I.N., Rudorfer, M.V., Linnoila, M., and Potter, W.Z.: Interacting neurotransmitter systems. A non-experimental approach to the 5HIAA-HVA correlation in human CSF. J. Psychiat. Res. 20: 175-193, 1986.

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In Press

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00428-08 LCS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protein Carboxyl Methylation: A Post Translational Modifier of Protein Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.M. Saavedra, Chief, Unit on Preclinical Neuropharmacology/LCS/NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been combined with Z01 MH 00433-07 LCS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01850-10 LCS
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PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Clinical pharmacology of antidepressants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)
William Z. Potter, M.D., Ph.D., Chief, Section on Clinical Pharmacology,
Laboratory of Clinical Science, NIMH

COOPERATING UNITS (if any)
Clinical Psychobiology Branch; Clinical Neuroscience Branch; and Laboratory of
Clinical Studies, NIAAA

LAB/BRANCH
Laboratory of Clinical Science

SECTION
Section on Clinical Pharmacology

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS: 5.2	PROFESSIONAL: 3.6	OTHER: 1.6
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CHECK APPROPRIATE BOX(ES)
 (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The therapeutic mechanism of action of antidepressant medications in humans remains unknown. Comparison of effects on specific neurotransmitters and their metabolites in cerebrospinal fluid (CSF), plasma and urine in the same patients continues. Findings of special interest during the last year include the following:

1. Unique effects of electroconvulsive therapy (ECT) in humans continue to emerge: unlike all antidepressant drugs studied it does not reduce whole body norepinephrine (NE) turnover as measured in urine or NE and serotonin turnover as measured by MHPG and 5HIAA, respectively, in CSF. In fact ECT increases both 5HIAA and the dopamine (DA) metabolite, HVA in CSF.
2. Based on these clinical findings we carried out experiments on ECT in rats and found a selective increase in the D₁ subtype of DA receptor in substantia nigra and caudate. Since ECT has been reported to have therapeutic effects in mania, psychosis and Parkinsonism as well as depression these DA effects likely have clinical and mechanistic implications.
3. In keeping with the theme that more than one neurotransmitter change is involved in antidepressant action, we have found that drugs with specific classes of initial biochemical effects have unique in vivo profiles of effects in humans only when changes of MHPG, 5HIAA and HVA are simultaneously taken into account. We have employed a 3-dimensional graph to obtain clear discrimination between five classes of drugs using these amine metabolites.
4. Alprazolam, a potent anti-anxiety agent with possible antidepressant properties, produces unusually robust decreases of ACTH and cortisol following intravenous administration. This finding may provide for a new test for the responsiveness of the HPA axis; i.e. is it more difficult to suppress in certain psychiatric illnesses? 293

Other Professional Personnel:

Matthew Rudorfer	Senior Staff Fellow	LCS/NIMH
John Hsiao	Medical Staff Fellow	LCS/NIMH
Emile Risby	Guest Researcher (NRSA)	LCS/NIMH
Ivan Mefford	Special Expert	LCS/NIMH
Laura Fochtmann	PRAT Fellow	LCS/NIMH
Markku Linnoila	Chief	LCS/NIAAA
Dennis Murphy	Chief	LCS/NIMH
Thomas A. Wehr	Chief	CP/NIMH
David Sack	Senior Staff Fellow	CP/NIMH
David Jimerson	Chief, Section on Biomedical Psych.	LCS/NIMH
David Pickar	Chief, Section on Clinical Studies	NSB/NIMH
Judith Rapoport	Chief, Child Psychiatry Branch	CHP/NIMH
Trey Sunderland	Senior Staff Fellow	LCS/NIMH

Project Description:

Our central aim is to understand the effects of major somatic anti-depressant treatments on the monoamine neurotransmitter systems in man. Systematic studies of drug action in normal volunteer controls and depressed patients controlling for pharmacokinetic and pre-drug physiologic variance has permitted demonstration of both predicted and unexpected biochemical alterations following treatment with drugs or convulsive therapy having widely differing acute primary effects.

Comparison of biochemical effects in CSF, plasma and urine in the same patients is continuing with new, efficient high performance liquid chromatography assays, and, when coupled with physiologic, behavioral and neuroendocrine measures, allows for clearer systems interpretations of changes. State-of-the-art measures of norepinephrine (NE), serotonin (5HT), dopamine (DA) and their metabolites are made under controlled conditions both cross sectionally in time and longitudinally in order to identify interrelationships, to test assumptions about the regulation of these neurotransmitter systems, and therefore to definitively describe effects of antidepressants as they relate to these neurotransmitter systems.

Methods:

The neurotransmitter systems of patients with either unipolar or bipolar major affective disorder are characterized after at least a 3-week drug-free period and then between the 3rd and 5th week following antidepressant treatment. Certain parameters, such as urinary transmitter and metabolite concentrations, are studied repeatedly following the beginning of each treatment. Patients admitted at steady-state of an antidepressant drug are also studied serially during the withdrawal phase. Parallel studies are performed in healthy volunteers and animal models when feasible as described below.

Treatments are ideally administered so as to produce maximal effects on the presumed target biochemical system such as inhibition of NE uptake after desipramine (DMI), of 5HT uptake after clomipramine (CMI), and of

MAO-Type A after clorgyline using control of pharmacokinetic variance (blood levels of DMI, CMI and desmethyl CMI) or biochemical indices (MHPG decrease after clorgyline). In the case of lithium and ECT, standard regimens are followed.

Novel putative antidepressants with no clear biochemical specificity such as alprazolam are also studied. Biochemical effects are studied after acute and chronic dosing, and, in the case of ECT, serially (generally weekly) throughout the course of treatment.

Studies in college age volunteers housed on the unit are of shorter duration (up to two weeks of active drug) and include DMI, lithium, and, in single intravenous doses, alprazolam.

Specialized pharmacokinetic and baseline biochemical studies are performed in volunteers age- and sex-matched to our accumulated patient population. These volunteers come to the clinic on the day of the study or are admitted for an overnight accommodation to the research unit.

Analysis of NE, 5HT, DA and their metabolites is carried out as described in a separate report, Z01 MH 01855-03 LCS. Using radiolabelled iodocyanopindolol as an antagonist lymphocyte beta receptor parameters are determined with ligand analysis of complex agonist displacement curves both before and after selected treatments.

To elucidate the mechanisms of action of ECT, we are administering ECS to rats (80 mAmp x .5 sec via earclip electrodes every other day for a total of 8 treatments) and assessing its effects from a number of vantage points. In particular, we have applied conventional membrane binding assays and quantitative autoradiographic techniques to examine ECS induced changes in a variety of receptor types. We are also obtaining micropunch specimens from brain and collecting 24 hour urines for assessing regional and total body catecholamine concentrations and turnover. Using the technique of tail artery cannulation which permits serial sampling of blood in unstressed freely moving rats, we have been able to conduct neuroendocrine challenge tests (paralleling those done on the clinical unit) in ECS treated rats. In addition, the cannulated rat model is ideal for assessing the acute effects of ECS on the neuroendocrine system.

Findings to Date:

1. Our ongoing treatment protocol with electroconvulsive therapy (ECT), which has now yielded some paired data in 15 patients, demonstrates important differences between the actions of convulsive therapy and those of antidepressant drugs. In contrast to every other effective antidepressant pharmacological treatment which we have studied, ECT does not reduce whole-body norepinephrine (NE) turnover. ECT does consistently lower basal plasma NE concentration by an average of 30%, but plasma NE measured after an orthostatic challenge also falls by an equivalent amount after the course of treatment, leaving the initial relative noradrenergic hyperactivity unchanged, despite clinical improvement in all but one patient.

Thus, ECT is unique in its spectrum of effects on the NE system raising the possibility that effects on other neurotransmitters may play a greater role than hitherto supposed (see below).

2. CSF monoamine metabolite concentrations also show a unique pattern of alteration after ECT, with little average change in MHPG but substantial rises in 5HIAA (not observed after any chronic antidepressant drug treatment) and even more so in HVA (by 24% and 35%, respectively). However, preliminary investigations into the responsiveness of the serotonergic system, using the prolactin stimulation by an intravenous challenge of clomipramine as a pharmacological role before and after a course of ECT, are negative. These findings suggest that dopaminergic effects of ECT may be important, a possibility which is suggested by preclinical findings summarized below.

3. Using conventional membrane binding techniques we have been able to confirm previous reports of decreased α_2 and β binding after ECS. Using [3H]-ketanserin we have found no significant differences in 5HT₂ receptor numbers or in 5HT₁ receptors or uptake sites. Despite previous negative results from other laboratories which utilized conventional binding techniques, by employing quantitative autoradiography we have found increases in D₁ receptors in substantia nigra pars reticulata, caudate and accumbens and corresponding changes in [3H]-forskolin binding which suggests parallel changes in adenylate cyclase. Autoradiographic studies of receptor binding in organs other than brain have revealed a decrease of β receptors in the heart ventricles, the first such demonstration of which we are aware after any antidepressant treatment. With our cannulated rat model we have been able to verify the expected increases in PRL levels with ECS itself. Consistent with the lack of change we find in serotonin receptors after ECS, there is no difference in the PRL response to citalopram (a serotonin uptake inhibitor) in ECS vs. sham treated rats. Coupled with our clinical investigations these animal studies point to the possibility of some important interaction of norepinephrine and dopamine systems in the mode of action of ECT.

4. Cerebrospinal fluid measures continue to prove useful in characterizing drug effects. In addition to individual monoamine metabolite concentrations, their relationships to one another are under study as we build upon our earlier investigations of drug effects on the noradrenergic system. The ratio of CSF HVA/5HIAA undergoes predictable changes with biochemically different antidepressants. In collaboration with Dr. Hans Agren, a former Visiting Fellow now in the Department of Psychiatry of Uppsala University in Sweden, we have examined results from several separate Swedish and American studies on three serotonin uptake inhibitors (zimelidine, clomipramine and citalopram), two norepinephrine uptake inhibitors (nortriptyline and desipramine), a dopamine uptake inhibitor (bupropion) and a dopamine agonist (bromocriptine) as well as two MAO inhibitors (clorgyline and deprenyl). In brief, serotonin uptake inhibitors all consistently increase the HVA/5HIAA ratio, norepinephrine and dopamine uptake inhibitors do not affect it whereas MAOI inhibitors and the dopamine agonist reduce the ratio. Furthermore, if drug effect is analyzed in terms of effects

on all three neurotransmitter metabolites (HVA, 5HIAA and MHPG) which can be displayed as a three dimensional plot, the values cluster according to type of treatment. Thus, we have established two methods of classifying biochemical drug effects in man that clearly discriminate classes which were not able to be discriminated using changes in amine metabolites taken singly.

5. We have continued our investigation of the serotonin reuptake inhibitor, intravenous clomipramine (CMI), as a pharmacologic probe of the serotonin system in depressed patients and healthy volunteers before and after ECT and lithium, respectively. Using low (10-12.5 mg) doses of CMI which produce no desmethyl CMI and minimal nausea in subjects, this challenge is specific to the serotonergic system, resulting in stimulation of plasma prolactin ACTH and cortisol concentrations with no effect on growth hormone. As noted above, preliminary studies of depressed patients reveal no effect of ECT on CMI responses. Furthermore, lithium treatment in volunteers does not alter the hormonal responses to CMI. These findings suggest that if there are ECT or lithium induced alterations in serotonin function they cannot be demonstrated with this serotonergic challenge.

6. Chronic lithium (14 days at 0.6-0.8 meq/l) in volunteers had no significant effect on plasma MHPG, HVA or the NE response to an orthostatic challenge on whole body NE turnover. Preliminary results reveal no evidence of effects on lymphocyte and platelet adrenergic receptor parameters using membrane preparations. This does not conflict with the findings of reduced isoproterenol-stimulated cAMP in whole lymphocytes reported by other groups. Thus, lithium-induced alterations in models of post-synaptic signal transduction are not accompanied by any evidence of changes in pre-synaptic function or receptor regulation (i.e. NE turnover or release).

7. Our initial study of the effects of intravenous alprazolam (APZ) in normal volunteers has been completed. The most striking findings were that APZ is surprisingly potent in reducing plasma ACTH and cortisol while increasing growth hormone. In light of studies under way in Dr. Gold's group, the effects on the HPA axis may reflect a direct inhibition of CRF. If so, APZ could be developed into an alternate challenge of the HPA axis in patients.

Significance to Biomedical Research and to the Program of the Institute:

Understanding of the mechanism(s) of action of antidepressant treatments produces improved therapeutics, new drugs, tools for studying and investigating the underlying pathophysiology of depression and therefore, ultimately, provides the basis for prevention.

From a therapeutic point of view pharmacokinetic studies have been critical to removing problems related to inappropriate dosing. Moreover, the systematic study of biochemically selective (clorgyline) and novel, presumably less toxic agents (alprazolam), as well as ECT -- a 50-year-old but still poorly understood intervention -- provide treatments which are effective in many patients who do not respond to standard antidepressants.

Of ultimate importance is the continued finding that changes of the noradrenergic system are always involved in the action of somatic antidepressant treatments. Although simple deficit or excess catecholamine hypotheses of depression do not explain drug action, it seems clear that to understand the mechanism we must understand the role of NE, individually and as it interacts with other transmitter systems. This aspect of interactions between systems may provide a means of predicting antidepressant response which, if feasible, would have a major impact in clinical care of a large population of patients.

Proposed Course:

A. To further develop our new understanding of effects of ECS in discrete regions in rats we will expand our work in quantitative autoradiography to other receptor types (D2, Substance P, Neuropeptide Y). We will also use these techniques to examine ECS effects on ion channels (calcium and sodium), enzymes (ACE) and second messenger systems (forskolin). In parallel studies we will compare ECS-induced receptor changes with those associated with lithium, insulin coma and other antidepressant treatments.

B. As a bridge from animal to clinical studies we will explore effects of various antagonists of specific neurotransmitter systems on the neuroendocrine response to ECS itself. In a complementary approach central ECS induced changes will be correlated with peripheral measures (urinary catecholamines, lymphocyte receptors, receptors in other organs) since these latter measures may be obtained directly or indirectly in homones.

C. A new putative antidepressant and indirect facilitator of noradrenergic function will be studied in patients and volunteers. This drug, idoxoxan, is a selective alpha 2 antagonist which is also of interest as a possible means of enhancing learning during periods of stress. It will be of particular interest to understand the adaptive biochemical changes in humans which occur after chronic administration.

D. In light of our findings reported in Z01 MH 00447-18 that renal clearance may be an important determinant of plasma concentrations of at least one neurotransmitter metabolite, HVA, we will investigate drug effects on renal clearance of those substances of greatest interest for neuropsychiatric research. Lithium will be the first drug investigated in this manner since it is known to affect some components of kidney function.

Publications

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Weingartner, H., Rudorfer, M.V., and Linnoila, M.: Cognitive effects of lithium treatment in normal volunteers. Psychopharmacology. 86: 472-474, 1985.

Rudorfer, M.V., and Potter, W.Z.: The tricyclic antidepressants. In Cutler, N.R., and Narang, P.K. (Eds.): Drug Studies in the Elderly. Plenum Press, New York, 1986, pp. 167-187.

Rudorfer, M.V., and Linnoila, M.: Electroconvulsive therapy. In Johnson, F.N. (Ed.): Lithium Therapy Monographs, Vol 1: Lithium Combination Treatment. Karger, Basel, Switzerland, 1987, pp. 164-178.

Rudorfer, M.V., Linnoila, M., and Potter, W.Z.: Accidental antidepressants: Search for specific action. In Dahl, S.G., Gram, L.F., Paul, S.M. and Potter, W.Z. (Eds.): Clinical Pharmacology in Psychiatry. IV. Selectivity in Psychotropic Drug Action - Promises or Problems. Springer-Verlag, Heidelberg, 1987, pp. 157-166.

In Press

Agren, H., Nordin, C., and Potter, W.Z.: Antidepressant drug action and CSF monoamine metabolites: New evidence for selective profiles on monoaminergic interactions. In Proceedings of the 6th International Catecholamine Symposium. Dahlstrom, A. et al. (Eds.), Alan R. Liss, Inc., New York, N.Y. (in press).

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Golden, R.N., DeVane, C.L., Laizure, S.C., Rudorfer, M.V., Sherer, M.A., and Potter, W.Z.: Bupropion: The role of metabolites in clinical outcome. Arch. Gen. Psychiatry (in press).

Golden, R.N., Markey, S.P., Risby, E.D., Cowdry, R.W., and Potter, W.Z.: Antidepressants reduce whole-body norepinephrine turnover while maintaining 6-hydroxymelatonin output. Arch. Gen. Psych. (in press).

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Potter, W.Z., Rudorfer, M.V., Lesieur, P., Risby, E.D., and Linnoila, M.: Biochemical effects of selective 5HT-reuptake inhibitors in man. In Gastpar, M. (Ed.): Selective 5-HT Reuptake Inhibitors: Novel or Common Place Agents. S. Karger, Basel (in press).

Potter, W.Z., Rudorfer, M.V., and Linnoila, M.: Effects of antidepressants on NE and its metabolites in cerebrospinal fluid, plasma and urine. In Proceedings of the 6th International Catecholamine Symposium. Dahlstrom, A. et al. (Eds.), Alan R. Liss, New York, N.Y. (in press).

Risby, E.D., Hsiao, J.K., Sunderland, T., Agren, H., Rudorfer, M.V., and Potter, W.Z.: The effects of antidepressants on the HVA/5HIAA ratio. Clin. Pharmacol. Ther. (in press).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01855-03 LCS
PERIOD COVERED October 1, 1986, through September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Central Neurochemistry Service		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) William Z. Potter, M.D., Ph.D., Chief, Section on Clinical Pharmacology, Laboratory of Clinical Science, NIMH Ivan N. Mefford, M.D. Special Expert/LCS/NIMH Sanford P. Markey, M.D. Chief/AB/LCS/NIMH		
COOPERATING UNITS (if any) Section on Analytical Biochemistry and Section on Biomedical Psychiatry, LCS, NIH; Laboratory of Clinical Studies, NIAAA		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Clinical Pharmacology		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 4.2	PROFESSIONAL: 0.7	OTHER: 3.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) The central neurochemistry service functions as a centralized laboratory for analysis of neurotransmitters and metabolites in body fluids collected within the intramural program. routine analysis include <u>norepinephrine</u> in plasma urine and cerebrospinal fluid (CSF), <u>epinephrine</u> in plasma urine and CSF, <u>dopamine</u> and <u>dopamine sulfate</u> in urine, plasma and CSF, catecholamine metabolites, <u>HVA</u> , <u>MHPG</u> and <u>DOPAC</u> in CSF, plasma and urine, <u>serotonin</u> in platelets, platelet poor plasma and CSF and <u>5-HIAA</u> in plasma, CSF and urine. GC-MS assays are used for total urinary <u>norepinephrine</u> , <u>epinephrine</u> , <u>dopamine</u> , <u>VMA</u> , <u>MHPG</u> , <u>HVA</u> , <u>DOPAC</u> , <u>metanephrine</u> and <u>normetanephrine</u> . HPLC with amperometric detection is used for all other assays. Implemetation of microbore HPLC analysis using novel surfactant chromatographic approaches allows measurement of free catecholamines as well as serotonin in CSF. This allows examination of diurnal rhythms and of drug effects on these amines in extracellular fluid. Some 16,000 assays were performed on over 8,000 samples which were processed during the last year.		

Project Description:

The Central Neurochemistry Service provides a centralized analytical facility whose focus is the measurement of neurotransmitters and metabolites in physiological fluids generated by the clinical intramural research effort. Four technicians are presently provided by NIMH and one by NIAAA. These individuals perform routine assays and participate in development of new assays four of these use primarily HPLC while one performs assays and methods development on GC-MS in collaboration with the Section on Analytical Biochemistry (Dr. Markey).

Methods:

As noted above, the major analytical effort involves high performance liquid chromatography (HPLC) with electrochemical detection. Using recently developed reagents for separation of biogenic amines and microbore technology, selective detection of catecholamines at the 45 pg/ml level has been accomplished. Novel ion-pairing reagents allow "on column" concentration of samples (amines) eliminating tedious derivatization and extraction steps.

1. Amperometric detection when coupled to microbore HPLC offers significant (~50 fold) signal enhancement when compared to conventional HPLC or to coulometric detection.

2. Using a "non-eluting matrix" approach, biogenic amines can be selectively concentrated "on column" eliminating the necessity of derivatization and extraction.

3. HVA, 5HIAA and MHPG can be determined simultaneously in a single plasma extract. Separate assays for plasma 5HIAA and HVA were combined with the MHPG assay, eliminating separate sample preparation steps and analyses.

4. Extraction of plasma MHPG, normally the rate limiting step in this assay, was modified to double the sample load able to be processed.

5. Columns can be prepared "in house" via slurry packing which is a considerable savings in time and cost. This is now offered as a service to other NIMH laboratories.

Findings:

1. Using amperometric detection, we are able to quantitate concentrations of epinephrine and serotonin in the 100 femtomole/ml range in cerebrospinal fluid. These methods (a combination of 1 and 2) are now routinely applied to CSF samples. Epinephrine concentrations are routinely found to be 1-5 pg/ml while serotonin is usually found in the 10-100 pg/ml range.

2. Concentrations of NE, MHPG, HVA, DOPAC, and 5HIAA are measurable in the picomole/ml range in CSF and/or plasma by amperometric detection. Thousands of samples have been analyzed and the results are described in reports from the various clinical investigators using these assays.

3. Evidence has been accumulated demonstrating that only arterial blood is a suitable source for measurement of epinephrine for stress indices.

4. Resting plasma norepinephrine is highly correlated with CSF norepinephrine suggesting that plasma may provide a suitable single measurement.

Significance to Biomedical Research and to the Program of the Institute:

Neurotransmitter system function is implicated in major psychiatric illness, in behavioral medicine (e.g. responses to psychological and physiological stress) and in the mode of action of psychoactive as well as cardiovascular drugs. Improved methods for studying these neurotransmitter systems are crucial to understanding their operation in humans since adequate animal models or in vitro systems do not exist.

Only by fully and accurately quantitating neurotransmitters and their metabolites will it be possible to distinguish alterations of output vs those of metabolism and to relate amount to function. These techniques provide the best current hope of biochemically identifying individuals with psychiatric disease, at risk for such illness and/or most likely to respond to specific treatment.

Proposed Course:

With full-time professional direction of the laboratory, we plan to achieve the following over the next year:

1. Continue to assess the usefulness of plasma MHPG vs plasma norepinephrine and noremetanephrine as an index of noradrenergic function.
2. Measure free amines and metabolites in plasma and urine to study the renal clearance of these compounds.
3. Study the effects of various antidepressant therapies on CSF serotonin concentrations.
4. Assess the functional role of epinephrine formation in brain brain via CSF measurements in psychiatric populations and following drug intervention.

Publications

- Agren, H., Mefford, I.N., and Potter, W.Z.: Does serotonin turnover regulate dopamine turnover. New Biochemical evidence in man. In Shagass, C., Josiassen, R.C., Bridger, W., Weiss, K., Stoff, D., and Simpson, G.M. (Eds.): Biological Psychiatry 1985: Proceedings of the IVth World Congress of Biological Psychiatry. Elsevier Science Publishing Company, Inc., New York, 1986, pp.
- Agren, H., Mefford, I.N., Rudorfer, M.V., Linnoila, M., and Potter, W.Z.: Interacting neurotransmitter systems. A non-experimental approach to the 5HIAA-HVA correlation in human CSF. J. Psychiatr Res. 20: 175-193, 1986.
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- Pickar, D., Roy, A., Breier, A., Doran, A., Wolkowitz, O., Colison, J., and Agren, H.: In: Psychobiology of Suicidal Behavior, Annals, N.Y. Acad. Sci., 487, 189-196, 1986.
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- Roy-Byrne, P.P., Rubinow, D.R., and Linnoila, M.: Relation between plasma prolactin and plasma homovanillic acid in normal subjects. Neuropsychobiology 16: 85-87, 1986.

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van Kammen, D.P., van Kammen, W.B., Mann, L.S., Seppala, T., and Linnoila, M.: Dopamine metabolism in the cerebrospinal fluid of drug free schizophrenic patients with and without cortical atrophy. Arch. Gen. Psychiatry 43: 978-983, 1986.

Flament, M., Rapoport, J., Murphy, D.L., Berg, C., and Lake, C.R.: Biochemical changes during clomipramine treatment of childhood obsessive-compulsive disorder. Arch. Gen. Psychiatry 44: 219-225, 1987.

Gjerris, A., Sorensen, A.S., Rafaelsen, O.J., Werdelin, L., Alling, C., and Linnoila, M.: 5-HT and 5-HIAA in cerebrospinal fluid in depression. J. Affective Disord. 12: 13-22, 1987.

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Potter, W.Z., Rudorfer, M.V., and Goodwin, F.K.: Biological findings in bipolar disorders. In Hales, R.E., and Frances, A.J. (Eds.): American Psychiatric Association Annual Review: Volume Six. American Psychiatric Press, Inc., Washington, D.C., 1987, pp. 32-60.

Roy, A., Guthrie, S., Pickar, D., and Linnoila, M.: Plasma norepinephrine response to cold challenge in depressed patients and normal controls. Psychiatry Res. 21: 161-168, 1987.

Roy, A., Pickar, D., Linnoila, M., Chrousos, G.P., and Gold, P.W.: Cerebrospinal fluid corticotropin-releasing hormone in depression: Relationship to noradrenergic function. Psychiatry Res. 20:229-237, 1987.

Sunderland, T., Tariot, P.N., Cohen, R.M., Newhouse, P.A., Mellow, A.M., Mueller, E.A., and Murphy, D.L.: Dose-dependent effects of deprenyl on CSF monoamine metabolites in patients with Alzheimer's disease. Psychopharmacology 91: 293-296, 1987.

Virkkunen, M., Nuutila, A., Goodwin, F.K., and Linnoila, M.: Cerebrospinal fluid monoamine metabolite levels in male arsonists. Arch. Gen. Psychiatry 44: 241-247, 1987.

In Press

Agren, H., Nordin, C., and Potter, W.Z.: Antidepressant drug action and CSF monoamine metabolites: New evidence for selective profiles on monoaminergic interactions. In Proceedings of the 6th International Catecholamine Symposium. Dahlstrom, A., et al. (Eds.), Alan R. Liss, Inc., New York, N.Y. (in press).

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Potter, W.Z., Rudorfer, M.V., Lesieur, P., Risby, E.D., and Linnoila, M.: Biochemical effects of selective 5HT-reuptake inhibitors in man. In Gastpar, M. (Ed.): Selective 5-HT Reuptake Inhibitors: Novel or Common Place Agents. S. Karger, Basel (in press).

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Z01 MH 01855-03 LCS

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01860-01 LCS

PERIOD COVERED

October 1, 1986, through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of Epinephrine in Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Ivan N. Mefford, Ph.D.

COOPERATING UNITS (if any)

Section on Analytical Biochemistry and Section on Biomedical
Psychiatry, LCS, NIH; Laboratory of Clinical Studies, NIAAA

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.3

PROFESSIONAL:

0.3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

Epinephrine is the least prevalent catecholamine in mammalian brain, localized in the most primitive and medial aspects. Traditional approaches to studying epinephrine in brain have treated it primarily as a neurotransmitter. Over the past two years our view has changed, to consider epinephrine primarily as an extraneuronal metabolite of norepinephrine, thus functioning as hormone in forebrain. This has prompted the study of the role of epinephrine in sedation and intoxication and the implementation of equilibrium dialysis as a method for monitoring extracellular fluid (ECF) epinephrine content. Inhibition of the epinephrine forming enzyme, phenylethanolamine-N-methyltransferase (PNMT) produced a potent and long-lasting antagonism of both barbiturate and ethanol intoxication in rats. ECF epinephrine exceeds norepinephrine at baseline throughout the hypothalamus in the anesthetized rat, suggesting that epinephrine may act as the primary agonist as extrajunctional α_2 receptors in this part of the brain. Monitoring CSF epinephrine in rhesus monkeys demonstrates marked increases in epinephrine content during barbiturate infusion, consistent with this hypothesis.

Project Description:

The goal of this work is to understand and describe the metabolism and physiological of endogenously formed epinephrine in the mammalian brain. This project is being studied using several approaches.

Metabolism of epinephrine. Accumulated pharmacological data suggests that enzymatic formation in the hypothalamus is dissociated from the storage sites of epinephrine. Evidence suggests that this synthesis may occur in non-neuronal elements. I am examining the possibility that this synthesis occurs in glial cells, specifically astrocytes. This is being studied in both astrocyte cultures and astrocytes isolated from adult rat brain. Future studies will examine the properties of uptake of norepinephrine into these cells.

Pharmacological manipulation of epinephrine synthesis. Considering epinephrine as an extraneuronal metabolite of norepinephrine suggests that the physiologically relevant pool of epinephrine is found in extracellular space. This pool is actively modified by the release of norepinephrine from selected neuronal populations, particularly the projections of the A₁ and A₂ cell body groups. Epinephrine synthesis via PNMT should provide slow elevation of this pool, but a rather short time course for clearance following enzyme inhibition. Numerous pharmacological manipulations of noradrenergic release and reuptake and metabolism can be studied including MAO inhibitors, uptake inhibitors, alpha₂ receptor agonists and antagonists and inhibitors of epinephrine synthesis. The effects of these manipulations can be measured in extracellular fluid using equilibrium dialysis.

Methods:

Equilibrium dialysis. Dialysis probes are prepared to provide optimum recovery and regional selectivity. Dialysis tubing, 250 um in diameter is used to prepare probes as described by (Zetterstrom, Sharp) and Ungerstedt. Collected dialysates are analyzed for amines using microbore HPLC with amperometric detection. Further selectivity is obtained by using IGEPON T-77 as a chromatographic modifier.

All other tissue, plasma and/or CSF analyses are accomplished using published HPLC techniques.

Findings:

1. Extracellular fluid epinephrine exceeds norepinephrine in anesthetized rat.
2. CSF epinephrine increased markedly in response to barbiturate administration in awake, unanesthetized monkey.

3. Astrocytes cultured from foetal rat hypothalamus have epinephrine - synthesizing capacity.

4. PNMT inhibition provides marked prophylaxis against ethanol or barbiturate intoxication, but not anesthesia.

Significance:

Understanding the metabolism and functional significance of epinephrine in mammalian brain may provide a great deal of insight into the mechanism of action of several classes of drugs. If, as this research proposes, epinephrine is an extraneuronal metabolite of norepinephrine, any drug affecting norepinephrine release, reuptake storage and metabolism would affect the hormonal pool of epinephrine. It is proposed that one of the functions of epinephrine in brain is tonic regulation of the level of arousal and reactivity to sensory stimuli. Some evidence suggests that epinephrine synthesis is important in reward mechanisms. Consequently, epinephrine synthesis may be important in antidepressant efficacy. Our work, already completed, suggests an important role for epinephrine in intoxication and tolerance to sedative hypnotics.

Proposed Course:

Test the hypothesis that hypothalamic epinephrine is an extraneuronal metabolite of norepinephrine, primarily from A₁ and A₂ projections, by:

A) Assessing the actions of intoxicants, anesthetics and sedative hypnotics in awake unanesthetized animals on extracellular epinephrine, norepinephrine, dopamine and serotonin.

B) Assessing effects of classical adrenergic drugs, amphetamine, cocaine, tricyclics, neuroleptics and MAO inhibitors on extracellular epinephrine.

C) Studying synthesis of epinephrine and uptake of norepinephrine in non-neuronal brain cells.

Publications

Mefford, I.N.: Distribution of epinephrine in mammalian brain. Clin. Neuropharmacol. 9(4): 177-179, 1986.

In Press

Campbell, B.G., Bobker, D.H., Mefford, I.N., and Weber, E.: Both the sigma-receptor specific ligand (+)3-PPP and the PCP receptor-specific ligand TCP act in mouse vas deferens via augmentation of electrically evoked norepinephrine release. Eur. J. Pharmacol. (in press).

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00787-08 LCS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Mechanisms of Isolation Call in Squirrel Monkey (*Saimiri sciureus*)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. D. MacLean Intramural Research Scientist LCS, NIMH
 Other: J. D. Newinan Research Physiologist LCE, NICHD

COOPERATING UNITS (if any)

Laboratory of Comparative Ethology, NICHD

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.4

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There are indications that in the therapsid-mammalian transition changes in the auditory and vocal apparatus afforded audiovocal communication as a means of maintaining maternal-offspring contact and contact of members of a group. The just completed present project is part of an investigation concerned with identifying the cerebral representation of the separation call, a basic mammalian vocalization that serves the above noted functions. For this purpose, squirrel monkeys are tested for their ability to produce spontaneous calls in isolation before and after ablations of different parts of the brain. The present study has focused on the midline frontolimbic cortex, one of two cortical areas where stimulation elicits vocalization in monkeys. Evidence derived by the process of elimination indicates that the spontaneous calls depend on the concerted action of a continuous band of rostral limbic cortex comprising parts of areas 24, 25, and 12. Ablation of the midline frontal neocortex peripheral to this limbic zone is compatible with criterion performance in the production of the call. The present report also includes ancillary observations in regard to separation calls emitted while the subject is awakening from sodium pentobarbital (Nembutal) anesthesia, as well as following the intraventricular administration of oxytocin.

Project Description:

Objectives: Since the mammal-like reptiles (the antecedents of mammals) were probably egg laying, and since their auditory apparatus resembled that of lizards, these and other considerations suggest that they may not have engaged in parental care or audiovocal communication. In the therapsid-mammalian transition, two small bones of the jaw joint of the mammal-like reptiles became transformed into the malleus and incus of the highly tuned mammalian ear. Present evidence indicates that the separation cry is universal among mammals, serving initially to maintain maternal-offspring contact and then, later, contact of members of a group. Hence the separation cry perhaps ranks as the earliest and most basic mammalian vocalization. The present investigation is concerned with identifying the cerebral representation of the separation cry, using squirrel monkeys as subjects. The present phase of the work has focused on the rostral midline frontolimbic cortex, one of two cortical areas in monkeys where stimulation elicits vocalization.

Methods Employed: Subjects are squirrel monkeys two or more years of age and of either sex, belonging to the two main species characterized by the ocular patch as "gothic" and "roman" and having distinctive separation calls. The subjects are tested for their ability to produce spontaneous separation calls before and after bilateral ablation of respective parts of the frontal lobe. Since the monkeys are tested while isolated in a sound reducing chamber, such experimentally induced vocalizations are referred to as isolation calls. Criterion performance is the production of 20 or more calls during a period of 15 min. The presence or absence of alterations in the pattern of the call is demonstrated by spectrographic analysis.

Major Findings: The present phase of the study has been concluded with the submission of a full length report for publication. The results may be briefly summarized by reference to the accompanying Figs. 1 and 2. Figure 1A shows Rosabal's cytoarchitectural areal parcellation. For facilitating comparison of lesions, the other figures show numbers of Rosabal's areas inserted into millimeter squares conforming to the planes of the brain atlas. The cortical area critical for the spontaneous call may be inferred by the process of the elimination. Pregenuel lobotomy or lobectomy (vertical line in Fig. 1B, subjects SC-8 and X-5), but not prefrontal lobectomy (SC-7), resulted in failure to produce spontaneous isolation calls. The results of the pregenual lesions do not answer the question as to whether or not severance of connections with the midline or lateral cortex rostral to the lesion or the midline cortex caudal to the lesion accounts for the deficit. The findings in a fourth subject (shaded area in Fig. 1C, subject R-5) indicated that the cortex essential for the call is located on the medial frontal surface. A successive narrowing down of the lesion indicated that the spontaneous call depends on the concerted action of a continuous band of rostral midline limbic cortex comprising parts of areas 24, 25, and 12 (Fig. 2B). Elimination of all the midline neocortex peripheral to this zone (Fig. 2A) or lesions of parts of the limbic zone itself (e.g. Fig. 2D) had no enduring effect on the production of the call.

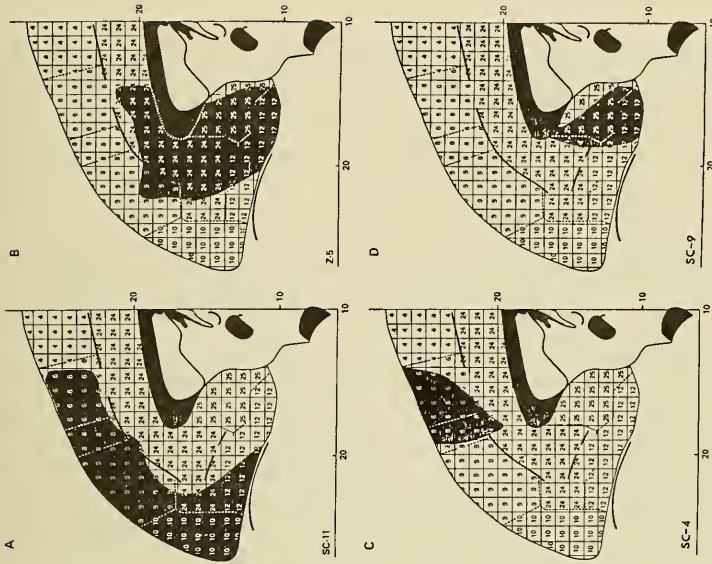


Figure 1

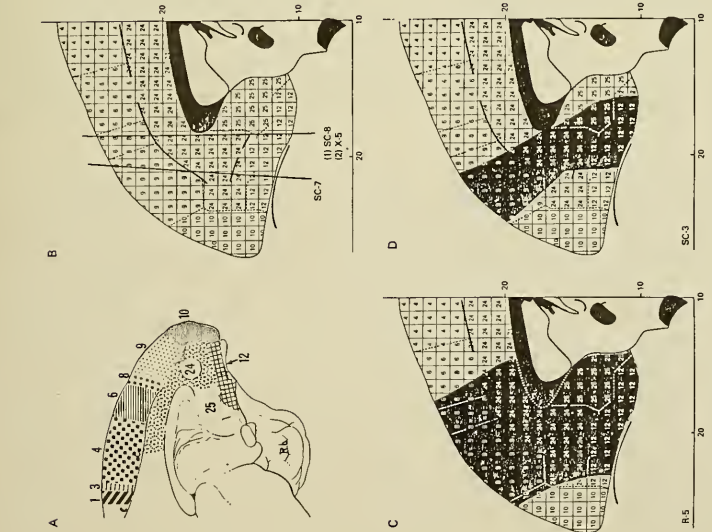


Figure 2

Ancillary Findings. Incidental to the present experiments, it has been noted that monkeys emit separation calls upon awakening from sodium pentobarbital (Nembutal) anesthesia. In the case of rodent pups, it has been reported that their ultrasonic separation calls may be induced by a decline in body temperature upon their removal from the nest (Okon, 1972). Recordings have been made on nine monkeys while awakening from pentobarbital anesthesia (P-084; Z-5; SC-7; SC-11; 1979; 1976; 368-P; 1991; 1989). Seven of nine subjects produced typical separation calls while awakening that persisted for periods ranging from 9 to 108 min. Peak calling ranged from 3.5 to 13 per min. For these pilot observations, conditions did not permit the control of ambient temperature. The findings in this small number of cases did not indicate that calling was initiated by a decline in body temperature. For example, one subject did not begin to call until its rectal temperature increased from 98° to 99°. It was observed in one case that one subject (1991) that emitted no isolation calls under usual conditions achieved a peak rate of nine calls per minute upon awakening from anesthesia. One subject (Z-5) in which midline frontolimbic ablation virtually eliminated the isolation call, produced calls at a rate of 3.5/min upon awakening from anesthesia. This finding suggests that calling during recovery from pentobarbital anesthesia depends on subcortical mechanisms, possibly at the midbrain level. Acetylcholine elicits vocalization when applied to the central gray matter, and it is known that acetylcholine is released during light stages of barbiturate anesthesia.

The adjuvant role of oxytocin in parturition and in milk secretion has long been recognized. Apropos of the nursing situation and the maintenance of maternal-offspring contact, it is of special interest that in a recent experiment performed with Gessa, the injection of 0.2 ug of oxytocin into the third ventricle of a squirrel monkey resulted in the production of separation calls that compared in rate and duration to what was observed in this same subject while awakening from pentobarbital anesthesia. Parenthetically, this monkey showed the manifestations of yawning, stretching, and penile erection that Gessa and co-workers (1987) observed upon injecting oxytocin into the paraventricular nucleus of the rat.

Significance to Biomedical Research and the Program of the Institute: Neurologists have frequently commented upon the persisting ignorance of specific cerebral mechanisms underlying laughing and crying. This lack of information is of major significance because in an ethnographic sense, the manifestations of crying and laughter would rank along with language as reflecting the evolution and status of the human condition. In preceding reports on this project it has been pointed out how the experimental findings, together with various clinical data, suggest that the evolution of the thalamocingulate division of the limbic system has partly involved the provision of a neural substrate for laughing and crying, as well as a reciprocal innervation of these two conditions. At the same time, attention has been called to the relevance of the research on the separation cry to such mental health problems as childhood separation anxiety, the "failure-to thrive" syndrome, grief reactions, depression (including premenstrual and post-partum), and various forms of addiction, particularly the addiction possibly determined by the high concentration of opiate receptors in the cingulate cortex. Functional anatomical aspects of the study are considered in the accompanying related project Z01 MH 00796-02 LCS.

Proposed Course: In view of the additional anatomical findings described in accompanying project Z01 MH 00796-02 LCS, it will be important to test not only the effects lesions of the various nuclei within the bounds of the internal medullary lamina, but also of the ventral anterior nuclei. The pilot observations in regard to separation calls evoked by the administration of pentobarbital deserve further investigation.

Publications:

Hotton, III, N., MacLean, P.D., Roth, J.J., and Roth, E.C.: The Ecology and Biology of Mammal-Like Reptiles. Washington, Smithsonian Institution Press, 1986, 326 pp.

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MacLean, P.D.: The triune brain. In Adelman, G. (Ed.): Encyclopedia of Neuroscience. Cambridge, Birkhauser Boston, Inc., (in press).

MacLean, P.D.: A reinterpretation of memorative functions of the limbic system. In Goldberg, E. (Ed.): Festschrift for Aleksandr Romanovich Luria. New York, The IRBN Press, (in press).

MacLean, P.D.: Anokhin's operational architectonics with respect to memory. In Sudakov, K. (Ed): Systems Research in Physiology, New York, Gordon and Breach, (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00796-02 LCS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cytochemical Tracing of Thalamic Connections with Midline Frontal Cortex

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. D. MacLean Intramural Research Scientist LCS, NIMH

Other:

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

0.6

PROFESSIONAL:

0.3

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In a parent project on the cerebral representation of the separation call (see Z01 MH 00787-08 LCS), it was found that ablation of the midline frontal cortex caudal to the polar area in squirrel monkeys did not (as was also known in the case of rhesus monkeys) result in discernible retrograde degeneration in the thalamus. The present project is employing cytochemical tracing techniques for obtaining clarification of this matter. Last year's report included a description of diverse thalamic nuclei, as well as extrathalamic structures, shown to be connected with different midline frontal areas. The present report focuses on additional findings suggesting an orderly projection of respective parts of the ventral anterior nucleus to different midline frontal areas. In the light of assorted experimental and clinical evidence that the thalamocingulate division of the limbic system and the striopallidonigral thalamic connections are implicated in crying and laughter, the anatomical findings suggest a link-up of mechanisms implicated in both the affect and expression of these manifestations.

Project Description:

Objectives: In a recently completed study on the role of the midline frontolimbic cortex (see accompanying report Z01 MH 00787-08 LCS) it was found in the squirrel monkeys used as subjects that except for the medial polar cortex, midline frontal ablations resulted in no clearly discernible retrograde degeneration in the medial dorsal nucleus or elsewhere in the thalamus. This finding was in agreement with long known observations that such lesions failed to produce such degeneration in rhesus monkeys. For example, in 1964 Akert wrote, "The existence in rhesus monkey of athalamic frontal areas is already suggested by the work of Walker (1938, Fig. 39)... [F]rontal granular cortex consists of two principal regions: one (lateral-ventral) which receives central projections from the medial dorsal nucleus, and another (dorsal-medial) which receives no essential projections from the thalamus and at most may be supplied by sustaining ones." In the present project, cytochemical tracing techniques are being employed to obtain clarification of this matter. Although the findings described in last year's report are in general agreement with what other workers have reported with respect to midline frontal connections with diverse thalamic nuclei, there are certain gaps and inconsistencies, particularly in regard to the ventral anterior nuclei, that, as will be explained, are especially important to resolve from the standpoint of clarifying mechanisms accounting for crying and laughing in human beings.

Methods Employed: Adult squirrel monkeys representative of the two main species (the so-called "gothic" and "roman" types) are used for these studies. The findings to date have been obtained by employing a modification of a technique utilizing wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP). The findings are now to be supplemented by other cytochemical tracing techniques that will help to resolve questions in regard to collateral innervation of midline frontal areas. The results of exploratory first trials with florescent dyes (fluorogold, rhodamine, fast blue) and silver protein are not yet available.

Major Findings: This report focuses on additional findings relating to the ventral anterior nuclei which include a lateral principal part (VApp) and a medial magnocellular part (VAMc). Generally speaking, the paragenual part of the rostral cingulate cortex and of the subcallosal cortex is identified with retrograde labeling of cells of VAMc that border upon the lateral part of the mammillothalamic tract and envelop the lower part of the tract, suggesting the appearance of a bed nucleus. In the frontal plane (c. AP 9.5-AP 9) where the two tracts appear above the level of the third ventricle, labeling of cells extends into the medial part of the ventral lateral nucleus (VLm). It is to be noted at this point that both VAMc and VLm receive afferents from the substantia nigra, whereas the principal part of VA receives projections from the internal segment of the globus pallidus. With injections of WGA-HRP further and further towards the frontal pole and then backwards in the neocortex to the rostral supplementary area, the labeling in the VA complex appears to extend further and further laterally as though, in a pictorial sense, one

were opening up a fan. With injections involving midline parts of areas 8 and 6 overlying the supragenual cingulate cortex, the greater part of VApp is labeled. WGA-HRP applied to these areas also results in labeling within the oral part of the ventral lateral nucleus (VLo). This nucleus, it should be noted, receives projections from the internal segment of the pallidum. The anterograde labeling seen with WGA-HRP method suggests that the frontal areas project back to the same nuclei from which they receive afferents.

Significance to Biomedical Research and the Program of the Institute:

As pointed out in last year's report, there is clinical evidence that the striopallido-thalamic circuits are involved in crying and laughter. There are also clinical indications that the thalamocingulate division of the limbic system is implicated in these same manifestations. Since the frontal lobes are known to play an important role in emotions and mood, the anatomical findings afforded by recent techniques reveal connections by which there may be a link-up between the striopallidonigro-thalamic complex that would serve as a substrate for both the affect and expression of crying and laughter. To be sure, the only direct overlap of the thalamic connections of the two systems in question are provided by VAmc and VLm. The nigra projects to both of these nuclei, while the the pallidum has some projections to VLm. However, there is evidence (Carmel, 1970) that VApp and VAmc are not only connected with each other, but also with intralaminar and other thalamic nuclei.

The question arises as to how the cerebellum would participate in mechanisms of crying and laughter, including the alternating waves of these manifestations. The cerebellar projections to the thalamus are described as having no overlap with the parts of VA and VL under consideration, nor is there clear evidence of intrathalamic connections (Asanuma et al., 1983). This is a question deserving further investigation, particularly in the light of cerebellar symptoms that may develop in patients with lesions of the premotor parts of the frontal lobes.

The anatomical findings in regard to VA help to clarify the observations by Starzl and Magoun and by Hanberry and Jasper some 35 years ago that this nucleus appeared to be central to a short-latency, diffuse projecting system affecting predominantly the frontal cortex, but also accounting for widespread cortical responses elsewhere. Hence the work of the present project is relevant not only to mechanisms of crying and laughter, but also to global functions of the frontal lobe, including functions depending on an integration of past memory, memory of ongoing experience, and a "memory of the future."

Proposed Course: To be continued with addition of techniques for demonstrating collateral innervation.

Publications:

MacLean, P.D.: The midline frontolimbic cortex in the evolution of crying and laughter. In Porecman, E. (Ed.): The Frontal Lobes Revisited. New York, The IRBN Press, 1987, pp. 121-140.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00797-02 LCS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Attachment

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. R. Insel Staff Physician LCS NIMH

Others: L. P. Miller Guest Worker LCS NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science, NIMH

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

0.5

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This is the second year of this project which investigates attachment and separation in infants and parents. Studies in rat pups have focused on the ultrasonic isolation call as a behavioral measure of separation distress. Following up on our earlier results demonstrating potent effects of the benzodiazepines on ultrasonic isolation calls during this year, we demonstrated increased occupancy of benzodiazepine receptors with in vivo receptor autoradiography during separation. These new results suggest a physiologic role for this receptor system in the separation response. We are currently testing this hypothesis further using intraventricular injections of endogenous benzodiazepine ligands into 1-week-old rat pups to investigate effects on the rate of isolation calls.

Our continuing studies of parental behavior have demonstrated that the brain is a target organ for the neuropeptide oxytocin and that oxytocin receptors increase in selected limbic regions during the postpartum period. As lesions in these regions slightly facilitate maternal behavior, oxytocin may inhibit rather than increase neural activity at these sites.

Project Description:

Objectives: We began this year with three objectives:

- (1) To investigate a physiologic role for the benzodiazepine receptor in the rat pup separation response.
- (2) To determine the significance of the oxytocin receptor increase in the postpartum period.
- (3) To extend our oxytocin-parental behavior studies to nonhuman primates.

Methods Employed: The infant response to brief separations has been investigated in rat pups from 1 to 14 days of age. Rat pups, when separated at these ages, emit ultrasonic calls which can be detected, quantified, and characterized using a computer-based sound spectrum analyzer. Our studies have investigated the pharmacologic modification of these calls by testing pups isolated for 2 minutes prior to subcutaneous drug administration, replacing the pup in its litter for 30 minutes, and retesting during a second 2-minute isolation period. In non-pharmacologic studies we investigated the influence of several other factors such as temperature, age, and presence of littermates on production of ultrasonic isolation calls.

In vivo labeling of benzodiazepine receptors involves injecting $^3\text{H-R0-15 1788}$ (3 Ci/pup) subcutaneously. Following injections, pups remain with their littermates or are separated for various periods of time. At 20 minutes postinjections, pups are sacrificed, and brains immediately removed and frozen. Frozen 20 sections are exposed to ^3H sensitive film for 8 weeks. The autoradiographic images are analyzed using a computer-based densitometric system. Nonspecific binding can be determined by pretreating a subset of pups with diazepam (5 mg/kg).

To determine the significance of the oxytocin increase in the postpartum, we embarked on a series of lesion experiments in 15-day pregnant females. Attempts at making cytotoxic lesions were not successful, probably due to the proximity of the target to the ventricle. Electrolytic lesions were more successful, using a radiofrequency lesion maker with an electrode placed stereotaxically into the bed nucleus of the stria terminalis. Maternal behavior was scored on the day of delivery and for 3 days postpartum by adapting a quantitative technique first published by Pedersen et al. (Science 216:648, 1982).

Parental behavior in pygmy marmosets was monitored and scored as noted in our previous annual report. Due to a decrease in the percentage of young raised successfully by our breeding pairs, this project was postponed until a more reliable baseline of parental behavior could be obtained.

Major Findings:

(1) Rat pups separated for 25 minutes from their littermates show a significant decrease in $^3\text{H-R0-15 1788}$ binding in frontal and cingulate cortex but not in several other cortical and subcortical regions examined. This decrease in binding probably results from release of an endogenous ligand which displaces $^3\text{H-R0-15 1788}$ from the receptor.

(2) Lesions of the bed nucleus of the stria terminalis do not decrease maternal behaviors and may, slightly increase some of the components of the maternal response. Taken together with our behavioral evidence that anosmia interacts with oxytocin's promaternal effects, these lesion data demonstrate that oxytocin's role may be to inhibit cannibalism or aggression towards the young as one part of a physiologic system of checks and balances.

(3) Pygmy marmosets remain a valuable research resource because of their pattern of maternal and paternal behavior; however, our current breeding colony will need considerable rehabilitation before invasive studies can be initiated.

Significance to Biomedical Research and the Program of the Institute:

Although the past decade has seen an explosion of research in the neurobiology of cognition, locomotion, and feeding, there has been a conspicuous absence of research into the neural substrates of such primary social behaviors as mother-infant attachment, pair-bonding, and affiliative behavior. This absence seems particularly noticeable in mental health research where the inability "to love and work" have long been recognized as a common feature of diverse forms of psychopathology and early experiences of loss or isolation have been shown to affect object relations in adulthood.

The demonstration that the same receptor which has been implicated in the pharmacologic modulation of anxiety is also activated physiologically during the infant's separation response provides the first biological evidence for Freud's dictum that "...anxiety proves to be a product of the psychic helplessness of the infant..." (1939).

Finally, this project has provided the first evidence for a morphologic change in brain with parturition--the significance of this change to postpartum mood and behavior remain to be assessed.

Proposed Course: The role of the benzodiazepine receptor in separation distress is currently being investigated further using direct intracerebroventricular injections of endogenous ligands into awake pups. In addition, other aspects of the separation response such as corticosterone and cardiovascular changes will need exploration in the coming months.

Maternal behavior continues to be an area of research excitement: the role of oxytocin receptors in males, the duration of the postpartum increase in brain oxytocin receptors, and the change in oxytocin content all need investigation. We will be extending our lesion studies to oxytocin cell

bodies and extending preliminary oxytocin immunohistochemical studies to an oligo-deoxyribonuclease probe for oxytocin mRNA using in situ hybridization.

We hope to return to the pygmy marmosets for studies involving ICV administration of oxytocin to determine if this peptide affects paternal as well as maternal behavior. This research awaits a more stable period of normative parental behavior in our colony.

Publications:

Insel, T.R.: Postpartum increases in brain oxytocin binding. Neuroendocrinology 44: 515-518, 1986.

Insel, T.R., Hill, J.L., Mayor, R.B.: Rat pup ultrasonic isolation calls: Possible mediation by the benzodiazepine receptor complex. Pharmacol. Biochem. Behav. 24: 1263-1267, 1986.

Insel, T.R., and Hill, J.L.: Infant separation distress in genetically fearful rats. Biol. Psychiatry 22:705-707, 1987.

Wamboldt, M.Z., and Insel, T.R.: The ability of oxytocin to induce short latency maternal behavior is dependent on peripheral anosmia. Behav. Neurosci. 101:439-441.

Insel, T.R.: The biology of parenthood. Amer. Health (in press).

Insel, T.R., Miller, L.P., Gelhard, R.E., and Hill, J.L.: The neural basis of the rat pup ultrasonic isolation calls. In Newman, J.D. (Ed.): The Physiologic Control of Mammalian Vocalization. New York, Plenum Press, in press.

Wamboldt, M.Z., Gelhard, R., Insel, T.R.: Gender differences in caring for infant *Cebuella Pygmaea*, role of infant age and relatedness. Dev. Psychobiol. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00798-01 LCS

PERIOD COVERED

February 1, 1987 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on the Development of the Cerebral Cortex

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. B. Stanfield Special Expert LCS NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science, NIMH

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIHAC, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

0.57

PROFESSIONAL:

0.40

OTHER:

0.17

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project comprises ongoing studies previously supported extramurally which are being continued in the LCS as well as studies initiated since the PI joined the LCS on February 1, 1987. This work on the development of the cerebral cortex which relies heavily on neuroanatomical techniques focuses on the role of eliminatory events which occur during normal brain development. Much of our effort has concentrated on the transient occipital cortical component of the pyramidal tract which we previously identified. We have recently found that in animals in which the occipital cortex receives an induced aberrant somatosensory input through the lateral geniculate nucleus as a result of neonatal enucleation and rostral cortical lesion, some occipital neurons will maintain a spinal projection. In addition, we have recently identified axonal eliminatory phenomena which occur during the development of the fornix, of the projections of the locus coeruleus, and of the major ascending thalamic afferent systems. Finally, we have continued our studies of the projections extended and maintained by heterotopic cortical transplants made during development. All of our findings in these experiments point to cortical locale as a decisive factor in determining which of the initially extended projections a cortical neuron will maintain.

Other Collaborative Professional Personnel Engaged on the Project:

D.D.M. O'Leary Assistant Professor Washington Univ. Sch. of Med., St. Louis, MO

Project Description:

Objectives: The overall goal of this project is to gain a better understanding of the development of the cerebral cortex. We have concentrated our studies on the role certain regressive or eliminatory phenomena play during normal cortical development. During the past ten years or so it has become increasingly clear that these are critical in shaping the projection patterns which are found in the adult cortex.

Methods Employed: The experiments completed or in progress can be grouped as five separate studies. These will be described separately:

1.) In an effort to determine what normally brings about the removal of the occipital pyramidal tract collaterals, we initiated a series of experiments involving neonatal lesions. We first removed the normal definitive targets of the occipital pyramidal tract neurons (which we had previously identified as the superior colliculus and the basilar pontine nuclei) to test whether the occipital pyramidal tract neurons would maintain their pyramidal tract collateral in the absence of their normal targets. The superior colliculus can easily be removed in young pups by aspiration, but this is not possible with the basilar pontine nuclei, not only because of their inaccessible position at the base of the brain, but also because of their proximity to the pyramidal tract itself which must be left intact in this experiment. Thus, the basilar pontine nuclei were removed indirectly. This could be accomplished by lesioning the cerebellum since this leads to a rapid and massive retrograde degeneration of the pontine nuclei. After the animals had grown beyond the stage at which the transient occipital pyramidal tract projection is normally eliminated, the retrogradely transported fluorescent marker, Fast Blue was injected into the pyramidal decussation and the occipital cortex was examined for the presence of any residual pyramidal tract neurons.

In a separate series of experiments we tested for the maintenance of occipital pyramidal tract axons in animals which at birth had received bilateral enucleations as well as lesions of the rostral one-half to two-thirds of the cerebral cortex. The rationale for this paradigm stems from recent observations we had made in eyeless mice (both congenitally eyeless and neonatally enucleated) with similar neonatal cortical lesions. We had found that in these mice the lateral geniculate nucleus comes to be innervated by medial lemniscal axons. We wondered if this would happen in these enucleated and lesioned rats and, if so, whether this somatosensory input to visual cortex could prevent the elimination of occipital pyramidal tract axons. Thus, when these neonatally lesioned and enucleated rats matured, we injected either Fast Blue into the pyramidal decussation or the anterograde tracer, WGA-HRP, into the dorsal column nuclei, and in some cases we did both injections.

2.) The results of the work described above emphasized the importance of the input relayed through the thalamus during development in determining the final projection patterns of the cortex. Yet outside of the visual system, little is known regarding the development of thalamic afferents. Thus, we have examined the development of the major ascending afferents to the thalamus in fetal and postnatal rats using TMB histochemistry following WGA-HRP injections into either the dorsal column

nuclei, the inferior colliculus, or the deep cerebellar nuclei, to label fibers of the medial lemniscus, the brachium of the inferior colliculus or the brachium conjunctivum, respectively.

3.) We have used both anterograde and retrograde tracing techniques to study the development of the fornix in rats. We undertook this study since incidental observations in material from an earlier study suggested that this primary efferent pathway of the hippocampal formation may exhibit a major transient component during development.

4.) In order to explore whether the phenomenon of collateral elimination occurs during the development of a brainstem nucleus, we injected Fast Blue into the spino-medullary junction of rats at various ages and examined the distribution of coeruleospinal cells within the locus coeruleus.

5.) Our observation that the distribution of pyramidal tract neurons is widespread during the first postnatal week and includes the occipital cortex, whereas no pyramidal tract neurons are found in the adult occipital cortex, led us to the suggestion that the differences seen in the projections of the various regions of the adult cortex are not intrinsic to the neurons found in these regions. Consistent with this idea is our subsequent finding that neurons within pieces of fetal occipital cortex which transplanted to the rostral cortex of a newborn host are able to extend pyramidal tract axons and maintain these beyond the age at which occipital pyramidal tract axons are normally eliminated. In order to explore further the projections extended and maintained by cortical neurons transplanted to a new cortical locale, we carried out additional experiments, transplanting rostral cortex to an occipital locale as well as occipital cortex to a rostral locale, and utilizing ^3H -thymidine autoradiography to identify the transplants and Fast Blue to examine the projections at different survival times and to additional targets.

Major Findings: The major findings of the studies described above can be summarized as follows:

1.) We have found that the early removal of the definitive targets of the transient occipital pyramidal tract neurons does not prevent the loss of these cells' pyramidal tract axons. However, many occipital cortical neurons can maintain pyramidal tract axons following neonatal enucleation and rostral cortical ablation. Further, this procedure induces an aberrant innervation of the dorsal lateral geniculate nucleus by medial lemniscal axons and the distribution and number of occipital neurons which maintain a pyramidal tract axon seem related to the location and magnitude of the induced lemniscal innervation of the lateral geniculate nucleus. These results not only demonstrate that this normally transient projection can be maintained, but underline the importance of the kind of thalamic input the cortex receives in influencing the projections which that cortex will maintain.

2.) We have found that by the day of birth, each population of axons of the major ascending afferents to the thalamus has already entered into and arborized within their appropriate thalamic relay nucleus. The overall distribution of each ascending afferent system, however, differs dramatically between young and mature rats. In neonatal rats, a substantial proportion of axons extend beyond the thalamus and often enter the internal capsule (many of these appear to bypass the thalamus altogether). In addition, axons which enter into and arborize within their appropriate terminal fields in the thalamus, frequently overshoot their targets and extend into adjoining thalamic nuclei. These early

overgrowths are all subsequently eliminated and the restricted adult distribution of each afferent system is evident by P30. Taken together with similar observations on the development of retinal fibers these results indicate that developmental overgrowths may be a general feature of the development of the major thalamic afferent pathways.

3.) Our study of the fornix indicates that these axons reach the caudal hypothalamus a day or two before birth. Before any fibers of the fornix can be identified entering into their principal target, the mamillary nuclei, a prominent contingent of fibers course past the mamillary complex. This postmamillary component continues to grow into the midbrain and pontine tegmentum during the first postnatal week as the projection into the mamillary nuclei is elaborated. During the second and third postnatal weeks, the postmamillary component of the fornix becomes progressively smaller until it is completely eliminated. The cells of origin of this transient postmamillary component of the fornix are found within the subicular complex of the hippocampal region. Most, if not all, of the cells of origin of the postmamillary component of the fornix survive the period during which this projection is eliminated. And recently, using a delayed double dye injection paradigm, we have shown that at least some of the subicular cells which transiently extend axons beyond the mamillary bodies maintain a projection to the mamillary bodies. Interestingly, the axons of the fornix which enter and eventually arborize within the mamillary nuclei and are maintained in the adult seem to arise during development as interstitial collaterals from parent fibers, the distal portions of which are subsequently eliminated. Further, the fact that although a postmamillary component of the fornix is not present in adult rats, such a pathway has been described in other species, such as cats, suggests that interspecific variations in projection pattern can result from the differential elaboration or elimination of an initially quite similar pattern of connections.

4.) Our observations on the development of the locus coeruleus indicate that coeruleospinal cells are present throughout the locus coeruleus just after birth, but are confined to its ventral portion by the end of the fourth postnatal week. We have shown that this change is not brought about by cell death, since neurons retrogradely labeled through their spinal axon following a neonatal injection of tracer are still present in the dorsal locus coeruleus even if the animal is not killed until the fourth postnatal week. Thus, the dorsal coeruleospinal neurons in newborn rats do not die but rather lose their spinal collateral. These results demonstrate that collateral elimination which we and others have repeatedly shown to occur during cortical development, may be a more generally occurring phenomenon than has previously been appreciated. Interestingly, in the locus coeruleus, as in the cortex, collateral elimination may be largely responsible for the spatial segregation of projection neuron populations which emerges during development as the adult pattern.

5.) Our observations on heterotopic cortical transplants made during development indicate that the projections which the cells in such transplants maintain are appropriate for the locale of the transplant rather than for the transplant site of cortical origin. This is true even though the transplanted tissue could be shown to initially extend transient projections, like those of the adjacent host cortex, to sites appropriate for its region of origin, but these were subsequently eliminated, while those projections to sites appropriate for the new cortical locale were maintained. These results are consistent with the notion that, at least as far as the connections they are able to maintain are concerned, the various regions of the neocortex may not be as distinct during development as might be thought. Rather, the distribution of cortical projection neuron populations seen in the adult does not seem to be "preprogrammed"

but results through a process of collateral elimination which restricts initially widespread distributions of projection neuron populations. Further, this restriction can be influenced by factors extrinsic to the cortical neurons themselves, such as their position within the tangential plane of the cortex.

Significance to Biomedical Research and to the Program of the Institute: Our studies on the eliminatory events that occur during brain development have helped to establish that these events constitute a major and widely present feature of the normal development of the central nervous system. In addition, these studies have helped to elucidate how such frankly regressive events may play critical roles in ensuring that development results in the establishment of appropriate neuronal connections.

Our work on the transient occipital pyramidal tract projection and our studies utilizing heterotopical cortical transplants suggest that the restriction of the initially widespread distributions of cortical projection neuron populations through collateral elimination allows the acquisition of regionally specific patterns of cortical projections without the necessity of these being prespecified to individual neurons. That is, individual neurons during development may be of a particular cortical projection neuron phenotype, but need not be intrinsically specified for the specific target appropriate for their position within the tangential plane of the cortex. In addition to limiting the amount of cellular prespecification necessary for normal cortical development, such stratagems introduce the potential for pliability and plasticity into the cortex during development and possibly during phylogeny as well.

Proposed Course: During the following year our work will proceed along the following lines:

We will continue and complete our studies of the projections extended and those maintained by heterotopic cortical transplants made during development.

We will continue and complete our analysis of the maintenance of the occipital pyramidal tract neurons and the medial lemniscal innervation of the lateral geniculate nucleus in neonatally lesioned and enucleated rats.

We will continue our delayed double dye study of the maintained targets of subicular neurons with transiently extend axons through the postmamillary fornix.

In order to explore what factors may be involved in the elimination of the postmamillary component of the fornix in rats, we will initiate a study to examine the fate of this projection in animals in which the mamillary bodies have undergone transneuronal degeneration following an early lesion to the cingulate cortex.

We will initiate a study to examine the distribution of locus coeruleus neurons with spinal projections and those with rostrally directed axons in tottering (tg/tg) mutant mice in which a hyperinnervation of some locus coeruleus targets in the absence of any increase in the number of locus coeruleus neurons has been reported.

Publications:

Chen, K.S., and Stanfield, B.B.: Evidence that selective collateral elimination during postnatal development results in a restriction in the distribution of locus coeruleus neurons which project to the spinal cord in rats. Brain Res. 410: 154-158, 1987.

Stanfield, B.B., Nahin, B.R., and O'Leary, D.D.M.: A transient postmamillary component of the rat fornix during development: Implications for interspecific differences in mature axonal projections. J. Neurosci. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00799-01 LCS

PERIOD COVERED

February 1, 1987 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Postnatal Neuronogenesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B.B. Stanfield Special Expert LCS NIMH

Others: T.R. Insel Staff Physician LCS NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science, NIMH

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIHAC, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

0.37

PROFESSIONAL:

0.27

OTHER:

0.10

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project comprises work previously supported extramurally which is being continued in the LCS as well as studies initiated since the PI joined the LCS on February 1, 1987. These studies have utilized ³H-thymidine autoradiography and neuroanatomical tract tracing techniques to study aspects of the continuing neuronogenesis in the adult dentate gyrus. We have found that dentate granule cells generated in the adult rat can extend axonal projections for appreciable distances, and we have initiated a study in pygmy marmosets to see if new granule cells are generated in an adult primate.

Project Description:

Objectives: We have studied the continuing neuronogenesis which occurs in adult rats in a limited segment of the cerebral cortex, the dentate gyrus. Until recently it was believed that neuronogenesis in mammals is completed before, or immediately after, birth, however, it is now clear that while this is true for the vast majority of neurons, in the rat dentate gyrus the production of granule cells continues at a slow rate well into adulthood and that cells generated in the adult may, in an older adult rat, account for almost half of the neurons present in the dentate gyrus. We are interested in learning more about this phenomenon with the eventual goal of understanding the mechanisms which control this slow accretion of neurons.

Methods Employed: Previous ^3H -thymidine autoradiographic studies in rodents have shown that while the full complement of neurons in most brain regions is produced prenatally, in a few sites, such as the dentate gyrus, the cerebellum and the olfactory bulb, the bulk of neuronogenesis occurs in the immediate postnatal period. More recent evidence indicates that after this perinatal surge in neuron production, dentate granule cells continue to be produced at a slow yet identifiable rate throughout most, if not all, of a rat's life. In order to determine whether the cells which incorporate the ^3H -thymidine in the adult rat dentate gyrus are in fact neurons which extend axonal projections, we injected a series of animals with ^3H -thymidine on postnatal day 100. Four weeks later we injected the retrograde tracer, Fast Blue, into the mossy fiber layer of the hippocampus, which contains the axons of the granule cells. After processing the sections from these brains for autoradiography, we examined them under bright- and dark-field illumination and fluorescence epi-illumination.

In order to determine if new granule cells are added to the dentate gyrus in adult primates, we have recently initiated a study utilizing cell counts as well as ^3H -thymidine autoradiography in the pygmy marmoset (*Cebuella pygmaea*). The pygmy marmosets were chosen due to the small brain and body size (an average adult weighs only about 150 g) and the relatively short period of time between birth and adulthood in these animals.

Major Findings: When we examined the sections from the animals injected with ^3H -thymidine on postnatal day 100, as expected we found an appreciable number of ^3H -thymidine labeled cells in the granule cell layer of the dentate gyrus. Further, many of these ^3H -thymidine labeled cells were labeled with the retrograde tracer as well. Thus, these ^3H -thymidine labeled cells are neurons which were generated on postnatal day 100 and which over the next thirty days extended an axon for a considerable distance. This not only demonstrates the remarkable ability of axons to grow and presumably to establish contacts within the normal adult neuropil, but also indicates that within the dentate gyrus this is a normally occurring ongoing process which results in the continuing addition of new-neuronal elements to the hippocampal circuitry.

Significance to Biomedical Research and to the Program of the Institute: These studies on the continuing neuronogenesis in the adult dentate gyrus have helped to establish that, in rodents at least, new neurons are generated in the adult, that these new neuronal elements do not simply replace neurons which are lost and that they are able to extend axonal processes and become integrated into the pre-existing circuitry of the

hippocampus. Further studies will hopefully help us to understand the role of this continuing neurogenesis in the function of the dentate gyrus and why, if new neurons can be continuously added here, does this not occur throughout the brain.

Proposed Course: During the following year our work will proceed along the following lines:

We will continue our study of axonal extension by adult generated dentate granule cell and in addition use various time periods between the ^3H -thymidine injection and the tracer injection to establish a time course for this axonal extension. This will provide us with information on the differentiation of these adult generated neurons which can then be compared with the differentiation of neurons generated during the early development of the dentate gyrus.

We will continue our study of the dentate gyrus of pygmy marmosets to determine if ^3H -thymidine will be incorporated by dentate granule neurons in adults of this species and if the total number of dentate granule neurons changes significantly during the lifetime of this primate.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02219-04 LCS

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Animal Models of Anxiety

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. R. Insel Staff Physician LCS, NIMH

COOPERATING UNITS (if any)

Laboratory of Comparative Ethology, NICHD; Addiction Research Center, NIDA, NIDA, Baltimore, MD

LAB/BRANCH

Laboratory of Clinical Science, NIMH

SECTION

Section on Comparative Studies of Brain and Behavior

INSTITUTE AND LOCATION

NIMH, NIH, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

0.5

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Our approach to the neurobiology of anxiety continues to be developmental with a focus on the role of early experience on the development of neurotransmitter and neuropeptide systems. We tested a specific hypothesis: that early stress activates brain corticotropin releasing factor (CRF) pathways and has long-term effects on brain CRF receptors with mixed results. As part of this project the development of CRF neurons, receptors, and second messenger was determined in fetal and postnatal rat brain. Receptors were found to be dramatically increased over adult levels soon after birth. Treating infants with CRF had short-term effects on development, long-term effects on behavior, but no lasting effects on brain CRF receptors. In related studies, neither noradrenergic lesions nor the behavioral paradigm of learned helplessness was found to alter brain CRF receptors.

Other Collaborative Professional Personnel Engaged on the Project:

E. DeSouza	Section Chief	ARC	NIDA, Baltimore, MD
T. Minor	Research Psychologist	UCLA	Brain Research Inst.

Project Description:

Objectives: This year was dedicated to testing a developmental hypothesis for the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis observed clinically with affective or certain anxiety disorders. It appears that during development there are critical periods (probably coinciding with the differentiation of neural elements) when ligands have organizational or "positive programming" effects on their receptor fields. For instance, postnatal administration of morphine to rat pups has been reported to induce an increase (not a decrease) in brain opiate receptors and to be associated with analgesia in adulthood. As loss of a parent has been associated with both affective and HPA axis dysregulation in adulthood, we reasoned that (a) early loss would increase endogenous CRF and (b) increases in CRF at a critical period in development would have long term effects on both behavior (response to separation in adulthood) and endocrine regulation (hyper-secretion of CRF with separation). The second part of this hypothesis was tested this year.

Methods Employed: Our first task was to define a critical period in development during which to intervene with exogenous CRF. We reasoned that the period of maximal expression of CRF receptors would provide such a period, as treatment during the normal overshoot of axons, dendrites, and cell bodies might protect against the elimination of postsynaptic elements. We studied CRF receptors by homogenate binding and in vitro receptor autoradiography in brains from rat fetuses 15, 17, 19, and 21 days old and from postnatal days 2, 8, 14, 21, and 28. Using a 32P-cAMP assay, we also investigated the linkage of the CRF receptor to its second messenger, adenylate cyclase. And, with a synthetic oligo-ribonuclease probe, we have used in situ hybridization to investigate the anatomy of brain CRF mRNA.

Based on results from these ontogeny studies, rat pups were injected with CRF (1 ug or 10 ug/day) from postnatal days 1 through 8. A subgroup of pups were tested with single peripheral or central (ICV) injections of CRF at day 8 to investigate acute behavioral effects. Outcome measures for adults injected as pups were open field tests, corticosterone response to CRF (3 ug or 10 ug/kg, IM), corticosterone response to separation (60 minutes), and brain and pituitary CRF receptor number.

Finally to examine whether CRF penetrated the blood-brain barrier in rat pups, the Oldendorf method was employed in 21 day old pups and in adults.

Major Findings:

- 1) CRF receptors appear in rat brain by fetal day 17, increase to more than 300% over the adult level by postnatal day 8, and reach adult levels by postnatal day 14. The cyclase generating complex itself

is relatively slow to mature - GTP, NaF, and forskolin (probes of different components within the adenylate cyclase complex) were not at their adult levels of effectiveness until postnatal day 14. CRF message was expressed as early as fetal day 17, with surprisingly high levels in the cortex.

- 2) After acute peripheral CRF injections in 8 day pups, a weak but significant increase in corticosterone was observed but no change in isolation calls was noted. Acute central injections were associated with a significant, dose-dependent decrease in ultrasonic isolation calls, with only a slight increase in corticosterone.
- 3) Chronic neonatal treatment with CRF was associated with early eye opening, increased exploratory behavior in the open field, no alteration in corticosterone response to CRF or separation, and an increase in pituitary but not brain CRF receptors.
- 4) CRF administered peripherally does penetrate the blood brain barrier of a 21 day old pup, but not in adult rats.

Significance to Biomedical Research and the Program of the Institute:

These studies provide a model with which to study how early experience might result in long term morphologic and behavioral effects. A careful chronologic characterization of the anatomic and functional ontogeny will be important for any such study to elucidate the rules by which ligands might influence the survival of their receptors during the period of postnatal sculpting of brain connections. In addition, the choice of neural system (CRF, benzodiazepine, excitatory amino acid) for study, will require an understanding of which systems are activated during those events in development which have long term consequences on behavior (see adjoining report on the Neural Basis of Separation and Attachment). Although our hypothesis regarding postnatal CRF, and adult depression can now be rejected, our results provide evidence that (1) early experience can alter behaviors relevant to exploration and (2) CRF treatment early in life may induce CRF receptors in the pituitary.

Proposed Course: During the coming year we plan to follow several leads from these studies. The ontogeny of second messengers in brain can now be investigated using labelled forskolin and phorbol ester (for adenylate cyclase and phosphoinositol proteins respectively). The role of excitatory amino acids in development will also be investigated in the coming months - first by describing the ontogeny of NMDA, kainic acid, and quisqualate receptors in brain, and then by treatments with excitatory amino acid antagonists (e.g. MK-801).

Publications:

Tamborska, E., Insel, T.R., and Marangos, P.: "Peripheral" and "central" type benzodiazepine receptors in Maudsley rats. Eur. J. Pharmacol. 126: 281-287, 1986.

Insel, T.R.: The neurobiology of anxiety: A tale of two systems. In Shaw, B.F., Segal, Z.R., Vallis, T.M., and Cashman, F.E. (Eds.): Anxiety Disorders. New York, Plenum Press, 1987, pp. 35-51, 1987.

Insel, T.R., Lane, E.A., Sheinin, M., and Linnoila, M.: Acute and chronic effects of desipramine administration to rhesus monkeys. Eur. J. Pharmacol. 136: 63-68, 1987.

Insel, T.R.: The development of CRF, CRF receptors, and receptor linkage to cyclase. In DeSouza, E., and Nemeroff, C. (Eds.): Corticotropin Releasing Factor. CRC Press (in press).

Marangos, P.J., Insel, T.R., Montgomery, P., and Tamborska, E.: Brain adenosine receptors in Maudsley reactive and non-reactive rats. Brain Res. (in press).

Wamboldt, M.Z., and Insel, T.R.: Pharmacologic models of anxiety. In Handbook of Anxiety. New York, Pergamon Press (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00382-13 LCS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.)

Localization and Characterization of Brain Neurochemicals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David M. Jacobowitz Chief, Histopharmacology Section LCS, NIMH
 Jaime Kapitulnik Visiting Associate LMC, NCI

COOPERATING UNITS (# any)

Laboratory of Molecular Carcinogenesis, National Cancer Institute

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

1.3

OTHER:

.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

(1) The presence of cytochrome P-450 in rat brain was studied by immunohistochemistry. Immunoreactive nerves were observed only in brain sections incubated with antiserum to 3-methylcholanthrene-induced cytochrome P-450. The most abundant concentration of nerve fibers with cytochrome P-450 immunoreactivity was observed in the globus pallidus. Immunoreactive fibers were also observed in the caudate-putamen, amygdala, septum, ventromedial nucleus of the hypothalamus, medial forebrain bundle, ansa lenticularis, and ventromedial portion of the internal capsule and crus cerebri. Cell bodies with cytochrome P-450 immunoreactivity were observed in the caudate-putamen and in the perifornical area of the hypothalamus. These results indicate that rat brain contains a form of cytochrome P-450 with antigenic relatedness to the hepatic 3-methylcholanthrene-inducible cytochrome P-450c. This cytochrome P-450 isozyme was detected in brain areas which metabolize morphine and convert estradiol and estrone into catecholestrogens, which suggest an important role for this enzyme in the metabolism of both exogenous and endogenous compounds in brain.

(2) A detailed immunocytochemical study of the localization of neuropeptides, enzymes and other neuoregulators in the dorsal tegmental region of the rat brainstem was carried out. Of the neurochemicals screened, atrial natriuretic factor (ANF), choline acetyltransferase (ChAT), cholecystokinin (CCK), calcitonin gene-related peptide (CGRP), dynorphin B (Dyn B), galanin (GAL), somatostatin (SOM), substance P (SP), neurotensin (NT), neuropeptide Y (NPY), vasopressin (VP), vasoactive intestinal peptide (VIP), serotonin (5HT), glutamic acid decarboxylase (GAD), and tyrosine hydroxylase (TH) were studied. The multiplicity of neurochemicals within this area suggests a possible influence on a variety of functions modulated by the lateral dorsal tegmental nucleus and other closely associated tegmental nuclei.

Project Description

Objectives: 1) To study the presence of cytochrome P-450 in the rat brain by immunohistochemistry.

2) To identify and localize a variety of neurochemicals within perikarya and fibers of the laterodorsal tegmental nucleus (ntdl) which is a cluster of cells located just medial to the locus coeruleus in the pontine brainstem.

Methods Employed: 1) Immunocytochemistry of cytochrome P-450. 2) Immunocytochemistry of ANF, ChAT, CCK, CGRP, Dyn B, GAL, SOM, SP, NT, NPY, VP, VIP, 5HT, GAD, TH.

Major Findings:

1) The most abundant concentration of nerve fibers with cytochrome P-450 immunoreactivity was observed in the globus pallidus of both normal and colchicine-treated rats. The caudate-putamen contained many extremely fine varicose fibers. Fibers were also observed in the amygdala, septum, ventromedial nucleus of the hypothalamus, medial forebrain bundle, ansa lenticularis, and ventromedial portion of the internal capsule and crus cerebri. Cell bodies with cytochrome P-450 immunoreactivity were observed in the caudate-putamen and in the perifornical area of the hypothalamus.

2) We have described a detailed study of the localization of neuropeptides, enzymes and other neuroregulators in the dorsal tegmental region of the rat brainstem.

Significance to Biomedical Research and the Program of the Institute:

1) The cytochrome P-450 isozyme was detected in brain areas which metabolize morphine and convert estradiol and estrone into catecholestrogens, which suggest an important role for this enzyme in the metabolism of both exogenous and endogenous compounds in the brain.

2) The multiplicity of neurochemicals within this area suggest a possible influence on a variety of functions modulated by the lateral dorsal tegmental nucleus and other closely associated tegmental nuclei.

Proposed Course of the Project: 1) The possibility that cytochrome P-450 is involved in the MPTP toxicity in monkeys which results in the Parkinson syndrome will be pursued. 2) Lesions of the lateral dorsal tegmental nucleus will be performed. The influence of these lesions on "boxing behavior" following prefrontal cortex injection of carbachol will be studied.

Publications:

Hamill, G.S., Skofitsch, G. and Jacobowitz, D.M.: Immunocytochemical localization of atrial natriuretic factor, galanin and calcitonin gene-related peptide within the rat interpeduncular nucleus. Brain Res. Bull. 17: 83-93, 1986.

Jacobowitz, D.M. and Skofitsch, G.: Calcitonin gene related peptide in the central nervous system: Neuronal and receptor localization, biochemical characterization and functional studies. In: Moody T. (ed.), Neural and Endocrine Peptides and Receptors. Plenum Publishing Co., 1986, pp. 247-288.

Kapitulnik, J., Gelboin, H.V., Guengerich, F.P. and Jacobowitz, D.: Immunohistochemical localization of cytochrome P-450 in rat brain. Neuroscience 20: 829-834, 1987.

Millan, M.A., Jacobowitz, D.M., Hauger, R.L., Catt, K.J. and Aguilera, G.: Distribution of corticotropin releasing factor (CRF) receptors in primate brain. Proc. Natl. Acad. Sci. USA 83: 1921-1925, 1986.

Zamir, N., Skofitsch, G. and Jacobowitz, D.M.: Distribution of immunoreactive melanin-concentrating hormone in the central nervous system of the rat. Brain Res. 373: 240-245, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00388-11 LCS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Coexistence of Peptides and Neurotransmitters

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David M. Jacobowitz	Chief, Histopharmacology Section	LCS, NIMH
Toshio Ohhashi	Guest Worker	LCS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.6

PROFESSIONAL:

1.2

OTHER:

.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Immunohistochemical studies have revealed that calcitonin gene-related peptide (CGRP) coexists with acetylcholine in motor cells of the spinal cord. Therefore this study was undertaken to investigate a possible interaction between the cholinergic nerve neurotransmitter and CGRP on neuromuscular transmission in the isolated rat diaphragm. Electrical stimulation of the isolated phrenic nerve resulted in twitch contractions which were dose-dependently potentiated by CGRP in concentrations ranging from 1.2×10^{-9} M to 3×10^{-7} M. The potentiating action of CGRP (3×10^{-7} M) returned to control level in about 25 min and was rarely tachyphylactic. The action of CGRP was dependent upon the stimulation pulse width ranging from 0.2 to 1.0 msec. Rat calcitonin (4.5×10^{-7} M) caused a minimal change in the amplitude of twitch contractions. CGRP had no effect on the quiescent striated muscle. Twitch responses to direct electrical stimulation was also enhanced by CGRP (6×10^{-8} M - 6×10^{-7} M) in the absence and presence of 10^{-5} M d-tubocurarine. These results suggest that CGRP modulates the action of acetylcholine at the motor-end plates of striated muscle. The possibility that an alteration in the normal peptide content in nerve endings (motor-end plates) of the body may lead to a variety of muscle malfunctions is of great clinical significance and should be studied in humans.

Project Description:

Objectives: To investigate a possible interaction between the cholinergic nerve neurotransmitter acetylcholine and calcitonin gene-related peptide (CGRP) on neuromuscular transmission in the isolated rat diaphragm.

Methods Employed: Electrical stimulation of the isolated phrenic nerve-diaphragm preparation.

Major Findings:

(1) Electrical stimulation of the isolated phrenic nerve resulted in twitch contractions which were dose-dependently potentiated by CGRP in concentrations ranging from 1.2×10^{-9} M to 3×10^{-7} M. The potentiating action of CGRP (3×10^{-7} M) returned to control level in about 25 minutes and was rarely tachyphylactic.

(2) The action of CGRP was dependent upon the stimulation pulse width ranging from 0.2 to 1.0 msec.

(3) CGRP had no effect on the quiescent striated muscle. Twitch responses to direct electrical stimulation was also enhanced by CGRP (6×10^{-8} M - 6×10^{-7} M) in the absence and presence of 10^{-5} M d-tubocurarine.

Significance to Biomedical Research and the Program of the Institute: The motor end plate in the diaphragm muscle is yet another example of the ever increasing reports of neuronal sites containing classical neurotransmitters coexisting with peptides. It would seem that acetylcholine and CGRP coreleased could serve to interact cooperatively to result in a potentiation of the muscle contraction. The demonstration that an increase in the pulse width results in a progressive increase in the contractile response following phrenic nerve stimulation suggests that the CGRP potentiating action may come into play when there is a need for greater muscle contraction. In this way the modulatory action of CGRP may serve to increase the capacity for the muscle contractile response to acetylcholine. The present results suggest that CGRP modulates the action of acetylcholine at the myoneural junction in striated muscle. This is of great physiological and clinical significance.

Proposed Course: Immunocytochemical work on the possible coexistence of CGRP and acetylcholinesterase (AChE), an enzyme present in motor-end plates will be studied in a variety of striated muscles and species including the human musculature.

Publications:

Crawley, J.N., Stivers, J.A. and Jacobowitz, D.M.: Neuropeptides modulate carbachol-stimulated "boxing" behavior in the rat medial frontal cortex. In Moody, T. (ed.), Neural and Endocrine Peptides and Receptors. Plenum Press, 1986, pp. 321-332.

Hamill, G.S., Skofitsch, G. and Jacobowitz, D.M.: Immunocytochemical localization of atrial natriuretic factor, galanin and calcitonin gene-related peptide within the rat interpeduncular nucleus. Brain Res. Bull. 17: 83-93, 1986.

Jacobowitz, D.M. and Skofitsch, G.: Calcitonin gene related peptide in the central nervous system: Neuronal and receptor localization, biochemical characterization and functional studies. In Moody, T. (ed.), Neural and Endocrine Peptides and Receptors. Plenum Press, 1986, pp. 247-288.

Sills, M.A. and Jacobowitz, D.M.: Chronic administration of either nialamide or desipramine decreases wet-dog shakes in rats produced by the TRH-analog MK-771. Brain Res. 401: 195-199, 1987.

Skofitsch, G. and Jacobowitz, D.M.: Quantitative distribution of galanin-like immunoreactivity in the rat central nervous system. Peptides 7: 609-613, 1986.

Skofitsch G., Sills, M.A. and Jacobowitz, D.M.: Autoradiographic distribution of ^{125}I -galanin binding sites in the rat central nervous system. Peptides 7: 1029-1042, 1986.

Zamir, N., Skofitsch, G. and Jacobowitz, D.M.: Distribution of immunoreactive melanin-concentrating hormone in the central nervous system of the rat. Brain Res. 373: 240-245, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00396-09 LCS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Study of Proteins Within the CNS by Two-Dimensional Gel Electrophoresis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

David M. Jacobowitz	Chief, Histopharmacology Section	LCS, NIMH
William E. Heydorn	Pharmacologist	FDA
Anne-Marie O'Carroll	Visiting Fellow	LCS, NIMH
Takemi Fukuda	Guest Researcher	LCS, NIMH
Lois Winsky	Guest Researcher	LCS, NIMH
Jitendra Patel	Visting Associate	BPB, NIMH

COOPERATING UNITS (if any)

Division of Neuropharmacological Drug Products, Food and Drug Administration

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MAN-YEARS:

3.4

PROFESSIONAL:

2.4

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)

The identification of interesting proteins within the CNS utilizing 2-dimensional gel electrophoresis (2DE) continues. 1) One of the largest proteins on our brain gels, "protein 36" has been isolated and purified from homogenates of rat brain. Protein 36 is a homodimer with a molecular weight of 64 kD and a monomeric weight of 37 kD with a pI of 6.5. Immunocytochemical studies using a rabbit antibody raised to this protein revealed that protein 36 is localized in large pyramidal cells, dendrites and axons of layer V of the cerebral cortex. 2) A second protein ("protein 94") has been isolated from the bovine brain and purified. This is a cAMP-stimulated phosphoprotein (M.W. 94 kD) which was originally identified in the rat neostriatum. Purification was monitored by autoradiography of ³²P-phosphorylated samples separated by SDS-PAGE. Immunocytochemical studies revealed that protein 94 was localized in astrocytes. 3) We have detected at least 3 Ca⁺⁺-binding proteins in the rat cortex by ⁴⁵Ca⁺⁺ autoradiography. Two proteins were identified as calmodulin and the B subunit of calcineurin. In addition, an unidentified Ca⁺⁺-binding protein of molecular weight about 18 kD and pI 5.4 was revealed. 4) Seven high affinity calcium binding proteins have been detected following ammonium sulfate precipitation. Three of the proteins have been identified (calmodulin, B subunit of calcineurin and vitamin D-dependent calcium binding protein). The identity of the other 4 proteins are unknown. 5) We have studied phosphoproteins present in seven discrete microdissected brain areas. Marked regional differences in the pattern of phosphorylation among the brain areas studied under basal conditions and following activation of cyclic AMP-dependent protein kinase and calcium/calmodulin-dependent protein kinase and protein kinase C were noted. 6) A series of studies examining proteins within auditory nuclei are under progress. Results indicate several differences in proteins visible on two-dimensional gels across auditory nuclei or more generally between sensory nuclei and other brain areas.

Project Description:

Objectives: 1) To isolate and purify a protein ("protein 36") that exists in high concentration on our brain gels. 2) To isolate and purify the 94 kD phosphoprotein and raise antibodies to the protein. 3) Identification of Ca^{++} -binding proteins using 2-DE and $^{45}\text{Ca}^{++}$ -autoradiography. 4) To study phosphoproteins in discrete regions of the brain using two-dimensional gel electrophoresis. 5) To study and compare proteins within different nuclei of the auditory system of rabbits and to determine whether Pavlovian conditioning training or daily exposure to auditory and pupillary stimulation produce any change in either the content or phosphorylation of proteins within auditory or other relevant nuclei in rabbit brain.

Methods Employed: 1) Two-dimensional gel electrophoresis; 2) Silver staining of proteins on polyacrylamide gels; 3) Electrophoretic transfer of proteins to nitrocellulose paper and subsequent identification of proteins by use of specific antisera; 4) Detection of Ca^{++} -binding proteins by $^{45}\text{Ca}^{++}$ -autoradiography; 5) Autofluorography of radiolabelled proteins. 6) $(\text{NH}_4)_2\text{SO}_4$ fractionation; 7) Biochemical separation techniques - ammonium sulfate precipitation; ion-exchange and gel filtration chromatography; 8) Immunization of rabbits for the production of antiserum; 9) Immunocytochemical methods; 10) Radiolabel phosphate (from ATP) incorporation into proteins from microdissected brain tissue; 11) Microdissection of discrete regions of the rat and rabbit brain; 12) Classical conditioning of the nictitating membrane response of rabbits.

Major Findings:

(A) We have isolated and purified a soluble protein designated "protein 36" which is found in the rat brain as one of the larger protein spots appearing on the 2DE gels. Protein 36 is a homodimer with a molecular weight of 64 kD and a monomeric weight of 37 kD with a pI of 6.5. Immunocytochemical studies using a rabbit antibody raised to this protein revealed that protein 36 is localized in large pyramidal cells, dendrites and axons of layer V of the cerebral cortex. The hippocampus contained cells in the stratum radiata and processes in the stratum pyramidale. A variety of cell types were also observed in the globus pallidus, thalamus and hypothalamus.

(B) Subcellular fractionation studies have shown the 94 kD protein to be localized predominantly to the cytosolic fraction. The pI value of the phosphorylated form of the denatured form of the protein was found to be approximately 4.7 while that of the unphosphorylated form of the protein was found to be approximately 3.8 by isoelectric focusing column chromatography. Production of a rabbit antiserum to the protein allowed immunohistochemical localization studies to be carried out. In the rat and monkey cortex grey and white matter astrocyte-like cells were observed. In the rat cerebellum, Bergmann fibers and cell bodies were observed in the molecular layer. In addition, processes which enveloped the purkinje cells were also seen.

(C) At least three Ca^{++} -binding proteins were detected in rat cortex by $^{45}\text{Ca}^{++}$ -autoradiography of two-dimensional electrophoretograms. The identities of two of these Ca^{++} -binding proteins were determined to be calmodulin and the B subunit of calcineurin. The identification was based upon the following

criteria: 1) comigration of polyacrylamide gels with the appropriate purified proteins, 2) staining of nitrocellulose blots with specific antisera for calmodulin and calcineurin and 3) ability to bind Ca^{++} . This information is useful in that it identifies two major brain proteins visible on silver-stained two-dimensional polyacrylamide gels. In addition, this data reveals the location of an unidentified Ca^{++} -binding protein of molecular weight $\sim 18,000$ daltons and pI 5.4 on these gels.

(D) A total of seven high-affinity calcium-binding proteins have been detected in rat brain. Of these seven proteins, three are detectable in a crude tissue punch of rat cortex while four are seen only after protein enrichment with ammonium sulfate. Three of the seven proteins detected in this study have been identified: calmodulin, the B subunit of calcineurin and the type II intestinal vitamin D-dependent calcium-binding protein. A fourth protein, soluble in a saturated solution of ammonium sulfate, is probably one of the subunits of the S-100 protein known to bind calcium. The identities of the other four proteins visualized by $^{45}\text{Ca}^{++}$ -autoradiography in this study are unknown. These results demonstrate that rat brain contains a number of high-affinity calcium-binding proteins. However, to consistently detect a number of these proteins, enrichment using differential protein solubility in ammonium sulfate is necessary.

(E) Phosphoproteins present in seven discrete microdissected brain areas have been studied using a combination of the micropunch technique, two-dimensional gel electrophoresis and autoradiography. Under basal conditions (no exogenous protein kinase activating factors added), about 40 discrete phosphoproteins were visualized over a pH range of 4.8-7.1 and a molecular weight range of 100,000-10,000 daltons. Approximately twelve of these labeled proteins correspond to silver-stained proteins visible on two-dimensional electrophoretograms of brain tissue. Three distinct regional differences in the basal pattern of protein phosphorylation were noted among the seven brain areas studied. Addition of calcium plus calmodulin to the incubation mixture markedly increased the phosphorylation of three acidic proteins in all seven brain areas studied. Activation of cyclic AMP-dependent protein kinase caused a qualitative increase in the degree of phosphorylation in all seven brain areas, with over fifty total proteins being labeled. Among the most prominent proteins visible after activation of cyclic AMP-dependent protein kinase were two poorly focusing proteins of molecular weight 94,000 and 83,000 daltons. Based upon physicochemical characteristics, a number of phosphoproteins labeled in this study have been identified. These results demonstrate that there are marked regional differences in the pattern of protein phosphorylation among microdissected areas of the rat brain. In addition, these data identify which silver-stained proteins visualized on two-dimensional electrophoretograms of brain tissue are phosphoproteins.

(F) A protein which appears to be specifically localized within several auditory and some other sensory nuclei was not seen in hippocampus, cortex, cerebellum or facial motor nucleus but was present in large amounts in the cochlear nucleus, inferior colliculus and trigeminal sensory nuclei. Lesions of the auditory nerve may decrease the content of this protein in cochlear nucleus. Another protein was detected which appeared to be specifically localized within the lateral superior olive. *In vitro* studies indicated marked variations in the degree to which phosphorylation was stimulated by the addition of 8-bromo

cyclic AMP or calcium plus calmodulin. For example, in the cochlear nucleus, only decreases in the phosphorylation of a few proteins were seen with the addition of the cyclic AMP analog. In contrast, increased radiolabel phosphate incorporation into protein was seen under this condition in the inferior colliculus.

Significance to Biomedical Research and the Program of the Institute: While many proteins remain to be identified certain important strides have been made in this direction. The information contained in this report will provide other workers in the field with a basis for reference and comparison. This is likely to accelerate the process of protein identification on 2DE gels and to enhance the value of this technique in the study of CNS proteins.

A major purpose of our investigation of proteins has been to establish immunochemically defined markers for neural cells and other cell structures. Underlying this approach has been the basic premise that antibodies can be valuable tools for studying a wide variety of biological systems including the nervous system. Measurement in blood and cerebrospinal fluid may provide new tools for diagnosis and monitoring of neurological diseases in addition to further immunohistochemical studies of pathological nervous tissue.

Astrocytes (glial) cells constitute a large fraction of the volume of the mammalian brain cortex. Much work needs to be done to unravel the multiple roles of astrocytes in brain function. The development of an antibody is significant in advancing our knowledge of astrocyte morphology and function in the brain.

Relatively little is known regarding the biochemistry of pharmacology of the auditory system. The results described in this report represent preliminary findings of a comprehensive study of auditory proteins and their phosphorylation. The identification of sensory specific proteins indicate the occurrence of unique biochemical events which may be functionally related to sensory processing. In addition, the examination of brain tissue from animals receiving auditory stimulation and/or Pavlovian conditioning may provide insights as to how a stimulus is coded within the brain and the degree to which this coding is dependent on its relevance to other environmental events.

Proposed Course of the Project: Future efforts will be directed at both identifying and learning more about the novel calcium-binding proteins and phosphoproteins. In addition, isolation and purification of unknown proteins will continue. We will set up cell cultures of astrocytes and study various types (fibrous, protoplasmic, oligodendrocytes) that react specifically with our astrocyte specific antibody.

Publications:

Fukuda, T. and Jacobowitz, D.M.: Purification and immunocytochemical detection of a protein that reveals layer V pyramidal cells in the rat cortex.. Brain Res., in press.

Heydorn, W.E., Creed, G.J., Creveling, C.R. and Jacobowitz, D.M.: Studies on catechol-O-methyltransferase in rat brain using two-dimensional gel electrophoresis. Neurochemistry International 8: 581-586, 1986.

Heydorn, W.E., Creed, G.J., Nguyen, K.Q. and Jacobowitz, D.M.: Effect of 5,7-dihydroxytryptamine on the concentration of individual proteins in different areas of the rat brain. Brain Res., 368: 193-196, 1986.

Heydorn, W.E., Creed, G.J., Patel, J. and Jacobowitz, D.M.: Distribution of proteins in different subcellular fractions of rat brain studied by two-dimensional gel electrophoresis. Neurochemistry International 9: 357-370, 1986.

Heydorn, W.E., Gierschik, P., Creed, G.J., Milligan, G., Spiegel, A. and Jacobowitz, D.M.: The β subunit of the guanine nucleotide regulatory proteins: Identification on two-dimension gels of brain tissue, existence of multiple charge forms and its regional and subcellular distribution in brain. J. Neurosci. Res. 16: 541-552, 1986.

Heydorn, W.E., Creed, G.J. and Jacobowitz, D.M.: Observations and implications on the migration of calmodulin in a 2 dimensional gel system. Electrophoresis 8: 251-252, 1987.

Narayan, R.K., Heydorn, W.E., Creed, G.J. and Jacobowitz, D.M.: Protein patterns in various malignant human tumors by two-dimensional gel electrophoresis. Cancer Res. 46: 4685-4694, 1986.

Narayan, R.K., Heydorn, W.E., Creed, G.J., Kornblith, P.L. and Jacobowitz, D.M.: Two-dimensional gel electrophoretic protein patterns in high grade human astrocytomas. In Walker, M.D. and Thomas, D.G.T. (eds.) Biology of Brain Tumour. The Netherlands, Martinus Nijhoff Publishers, 1986, pp. 7-14.

Rodriguez-Sierra, J.F., Heydorn, W.E., Creed, G.J. and Jacobowitz, D.M.: Isolation of specific proteins affected by estradiol in the arcuate-median eminence of prepuberal female rats. Brain Res. 399: 379-382, 1986.

Rodriguez-Sierra, J.F., Jacobowitz, D.M. and Blake, C.A.: Effects of neuropeptide Y on LH, FSH and TSH release in male rats. Peptides 8: 539-542, 1987.

Rodriguez-Sierra, J.F., Heydorn, W.E., Creed, G.J. and Jacobowitz, D.M.: Incorporation of amino acids into proteins of the hypothalamus of prepuberal female rats after estradiol treatment. Neuroendocrinology, in press.

Youdim, M.B.H., Sills, M.A., Heydorn, W.E., Creed, G.J. and Jacobowitz, D.M.: Iron-deficiency alters discrete proteins in rat caudate nucleus and nucleus accumbens. J. Neurochem. 47: 794-799, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00397-09 LCS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Autoimmune Aspects of Disease

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

James S. Frazier	Staff Fellow	LCS, NIMH
Hiroyasu Nakata	Visiting Scientist	LCS, NIMH
David M. Jacobowitz	Chief, Histopharmacology Section	LCS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.3

PROFESSIONAL:

1.1

OTHER:

.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Autoimmunity has been implicated in an increasing number of diseases including psychiatric disease. Using 2D gel electrophoresis and Western immunoblotting techniques, the study of CNS proteins continue as we look for specific proteins which might be target antigens for autoantibodies of pathologic origin. First, we have found that there exists a number of autoantibodies in the sera of normal animals and humans that react with brain cortical proteins. These include antibodies to: the cytoskeletal proteins actin, tubulin and GFAP; the enzymes sGOT and neuron specific enolase; and the β -subunit of the signal transducing G protein. Second, we have found there to be no obvious differences between the autoantibodies seen in normals and those seen in schizophrenics. Third, we have documented the presence in a small percentage of Type I diabetics of an antibody to a 25,000 MW pancreatic specific protein. Fourth, we have purified and partially characterized this protein. And fifth, we have raised a rabbit antibody that was shown by immunocytochemistry to specifically bind to islet cells of the monkey pancreas.

Project Description

Objectives: 1) To study the autoimmune aspects of diseases which might directly or indirectly affect the brain. 2) To characterize the "normal" immune state such that comparisons between normal processes and disease processes might be made. 3) To screen sera from schizophrenic to other psychiatric patients for disease specific autoantibodies. 4) Once target antigens for potentially pathogenic antibodies have been identified, to purify, characterize and study this protein in detail.

Methods Employed:

1) Two-dimensional gel electrophoresis. 2) Silver staining of proteins on gels. 3) Electrophoretic transfer of proteins to nitrocellulose paper and subsequent immunoblotting with sera or CSF. 4) Ultracentrifugation, ammonium sulfate fractionation, cation exchange chromatography. 5) Antibody formation via rabbit immunization. 6) Fluorescence microscopy.

Major Findings:

1. There exists in normal sera a number of autoantibodies with the capacity to react with CNS proteins. One to fifty dilutions of sera incubated with 2D Western immunoblots of cortical proteins revealed that 100% of 8 sera tested had an antibody to Actin, 75% to Tubulin, 75% to GFAP, 88% to sGOT, 100% to NSE and 63% to the β subunit of G proteins. Antibodies remained reactive out to 1 to 500 dilutions, there existed a number of immunoreactive proteins yet to be identified.
2. Schizophrenic sera (n=21) contained antibodies with reactivity no different from control, when sera samples were tested against cortical proteins at a 1:50 dilution.
3. Two out of 30 Type I diabetic sera contained an antibody to a 25,000 dalton human pancreatic specific membrane-bound tetraisomeric protein with pI values of approximately 7.0-8.0. No controls (n=12) contained this antibody. Positive diabetic sera showed reactivity out to a dilution of 1:2500, while controls were negative at 1:10 dilution. Positive sera was negative when tested against human liver, adrenal, thyroid, salivary, kidney and brain. Positive sera remained positive when reacted against human pancreas from six donors of differing age and sex. Positive sera was positive against immunoblots of cultured human islet cells, but negative against rat insulinoma cells.
4. When separated by ammonium sulfate fractionation and cation exchange chromatography, the purified pancreatic protein was injected into a rabbit. The resultant antibody was found to have immunochemical reactivity at a 1:3000 dilution with the islets of Langerhans of the monkey pancreas while pre-immune sera was negative. Interestingly, the sera showed immunoreactivity for the juxta glomerular apparatus of the rat kidney.

Significance to Biomedical Research and the Program of the Institute:

The finding that there are sera of normal control autoantibodies that bind proteins, including neuron-specific proteins suggests that alterations of the blood-brain barrier may make possible the entrance of the antibodies into the brain and thereby possibly potentiate, if not initiate various psychiatric and neurologic sequela. The inability to find schizophrenic specific antibodies may mean that autoimmunity is not a component of schizophrenia, or it may mean that non-cortical areas of the brain need to be examined. Also, sera and CSF samples taken during varying phases of altered cognitive states might need to be examined.

The finding of a Type 1 diabetic specific antibody and the characterization of it's target protein may provide valuable insights into the pathogenesis of this disease. More importantly, finding this antibody lends credibility to the use of 2D gel electrophoresis and Western immunoblotting techniques for the identification of potentially pathologic antibodies. Further refinement of these techniques for studies of CNS immunopathology may help define more clearly the role of autoimmunity in psychiatric disease.

Proposed Course of the Project: Further work will focus on attempts to obtain a sequence of the diabetic protein antigen in order to learn whether or not we are dealing with a known protein. Further work with the antiserum will be undertaken to do immunocytochemical studies on human pancreas and other organs (e.g., kidney).

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02377-01 LCS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Study of Adenosine Receptors: Isolation and Characterization

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Hiroyasu Nakata Visiting Scientist LCS, NIMH

David M. Jacobowitz Chief, Histopharmacology Section LCS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Isolation of A_2 adenosine receptors from rat brain membranes have been performed. The purification methods included solubilization by digitonin and column chromatographies using Q-Sepharose, hydroxylapatite, Green A-agarose and Mono Q. Approximately 1000-fold purification was achieved at the last stage of purification and the specific binding activity of the final preparation was ~ 100 pmol/mg protein assayed using [3 H]N-ethylcarboxamideadenosine (NECA) as a ligand. The apparent molecular weight of the partially purified A_2 adenosine receptors was estimated to be approximately 80,000 by HPLC (TSK 4000PW-5000PW) experiments.

It was also found that A_2 adenosine receptors could be separated from A_1 adenosine receptors or other unknown adenosine binding sites by hydroxylapatite chromatography.

Mouse mastocytoma P815 cell membranes were demonstrated to have A_2 adenosine binding sites in a relatively high concentration. These adenosine binding sites were also solubilized by detergents without any significant changes of ligand binding properties.

Project Description:

Objectives: 1) To isolate and characterize the biochemical properties of adenosine receptors from rat brain membranes. 2) To identify and classify adenosine receptors of mouse mastocytoma P815 cell membranes.

Methods Employed:

1) Ultracentrifugations for subfractionation of rat brain membranes and for isolation of solubilized receptor fractions. 2) Column chromatographies of ion-exchange resins, hydroxylapatite, or dye-coupled agarose gel. 3) FPLC and HPLC. 4) Radiolabeled ligand binding assay using cell harvester. 5) One- and two-dimensional polyacrylamide gel electrophoresis. 6) Intraperitoneal injection of mastocytoma P815 cells to DBA/2 mice to grow the cells and collect the ascitic fluid to harvest the grown cells.

Major Findings:

(A) Approximately 1000-fold purification of adenosine receptors which showed A_2 -type pharmacology was obtained after several purification steps, i.e., solubilization with digitonin, Fast Q- Sepharose chromatography, hydroxylapatite chromatography and Mono Q chromatography. The specific binding activity of the final receptor preparation assayed using [3H]NECA (20 nM) as a ligand was approximately 100 pmol/mg of protein. The apparent molecular weight of this partially purified A_2 adenosine receptor was calculated to be approximately 80,000 by HPLC (TSK 5000PW-4000PW, tandem-linked columns) experiments.

(B) A_1 and A_2 adenosine receptors could be separated from each other by hydroxylapatite chromatography. When solubilized preparations from rat brain which contained both A_1 and A_2 adenosine receptors was applied to a hydroxylapatite column and the column was eluted with a gradient of potassium phosphate, three major peaks which had adenosine binding activity were found (designated Peak A, Peak B and Peak C by the order of elution). Peak C was A_2 adenosine receptors and Peak A was A_1 adenosine receptors judging from their ligand binding pharmacology. Peak B was an unknown adenosine binding protein which should be examined further.

(C) Adenosine binding sites which showed A_2 -type pharmacology were identified in membranes of mouse mastocytoma P815 cells. The dissociation constant (Kd) was 380 nM and the maximum specific binding was 20 pmol/mg when assayed at 0°C using [3H]NECA as a ligand. The rank order of potency for inhibition of [3H]NECA binding was NECA > N-cyclopropylcarboxamide adenosine > 2-chloroadenosine > isobutylmethylxanthine > phenylisopropyladenosine, which was a typical pharmacology for A_2 -adenosine receptors. These adenosine binding sites were solubilized by either sodium cholate or digitonin. The solubilized binding sites retained the same adenosine binding characteristics as those of membrane-bound form. The apparent molecular weight of the adenosine binding sites solubilized with digitonin was estimated to be approximately 300,000 by gel filtration experiments.

Significance to Biomedical Research and the Program of the Institute: Adenosine and its stable analogs have pronounced physiological effects on various tissues including nervous tissue. These include modulation of adenylate cyclase, inhibition of both nerve cell firing and neurotransmitter release in vivo and in vitro and a sedative action thought to be centrally mediated. Most of these actions are thought to be mediated via the cell surface receptor to adenosine. Therefore, it is important to characterize the biochemical properties of the adenosine receptor to understand the function of adenosine which has significant pharmacological effects. These adenosine receptors are usually classified as A_1 and A_2 . A_1 adenosine receptors are linked to inhibition of adenylate cyclase whereas A_2 adenosine receptors are linked to activation of adenylate cyclase. Although it is important to isolate these receptors and study their biochemical functions, very little biochemical work, especially on A_2 adenosine receptors, has been done so far. As an initial step toward complete understanding of biochemical properties of adenosine receptors, A_2 -adenosine receptors were solubilized and partially purified up to 1000-fold using rat brain membranes as starting materials. During the purification steps, it was also found that A_1 , A_2 and the other unknown adenosine binding proteins could be separated from each other on hydroxylapatite chromatography. This finding will be useful as a convenient method for separation of A_1 and A_2 adenosine receptors from crude mixtures.

It is very important to find a good cell culture system for studying adenosine receptor functions in living cells. For that purpose, several cultured cells were screened. Mouse mastocytoma P815 cells which can be easily grown in both cell culture systems and in ascities fluid of DBA/2 mice have been known to secrete histamine and 5-hydroxytryptophan and such secretions are likely to be affected by adenosine suggesting the presence of adenosine receptors. From the ligand binding studies, it was demonstrated that P815 cell membranes had a high concentration of adenosine binding sites which showed A_2 type pharmacology. A_1 type adenosine binding sites were not identified. These results demonstrated that the mastocytoma P815 cell will be a good cell line for the study of functions of A_2 -adenosine receptors in culture systems.

Proposed Course: Isolation and purification studies will continue.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02239-03 LCS

PERIOD COVERED

October 1, 1986 to September 31, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Conceptual Analysis of Complex Biobehavioral Population Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

John B. Calhoun Chief URBS LCS NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Clinical Neuropharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

1.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated due to the retirement of the principal investigator.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 MH 00153-10 CHP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Treatment of Obsessional Children and Adolescents with Clomipramine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith L. Rapoport, M.D., Chief, CHP, NIMH
Henrietta Leonard, M.D., NRSA Fellow, CHP, NIMH
Dennis L. Murphy, M.D., Chief, LCS, NIMH
Theodore Zahn, Ph.D., Research Psychologist, LPP, NIMH
Agnes Whitaker, M.D., Columbia University
Susan Swedo, M.D., Staff Fellow, CHP, NIMH
Martha Denckla, M.D., Chief, Autism & Behav. Dis. Sec., DNB, NINCDS
Robert Freiland, M.D., LN, NIA

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, NIMH; Clinical Neuropharmacology Branch, NIMH; National Institute of Neurological & Communicative Disorders & Stroke; Columbia University

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.8

PROFESSIONAL:

2.3

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Obsessive Compulsive Disorder (OCD) is now recognized as a common mental disorder, occurring in perhaps three million people in this country alone. In adolescents, the prevalence maybe as high as one percent.

Follow-up studies in our Branch show a chronic course for the disorder with increasing severity of depression and anxiety over time. Clomipramine has a highly specific beneficial effect on obsessions while Desmethyylimipramine (DMI) another tricyclic antidepressant did not differ from placebo.

New studies with brain imaging (PET and CT scan), as well as a survey of patients with Sydenham's chorea, implicate abnormalities in the basal ganglia while spinal fluid studies show that low concentration of CSF 5-HIAA, the major serotonin metabolite, is associated with more severe OCD.

Project Description:

Objectives: To examine the biological correlates, treatment, and natural history of Obsessive Compulsive Disorder (OCD) in childhood and adolescence.

Methods Employed: A comparison of clomipramine (CMI) and desmethylimipramine (DMI) treatments of adolescents with Obsessive Compulsive Disorder is ongoing. After a baseline observation week, there are two weeks of single blind administration of placebo followed by double blind crossover administration of clomipramine or desmethylimipramine, each for five weeks. To date, 25 children and adolescents have completed this protocol. Most have agreed to a lumbar puncture during the baseline procedure.

Spinal fluid samples are being sent for immunoglobulin, as well as CSF monoamines and metabolites, to compare with controls and relate to clinical measures.

A survey of adolescents who had recently had Sydenham's chorea or Rheumatic fever alone without accompanying Sydenham's chorea, has been initiated. Subjects were surveyed with the Leyton Obsessional Inventory-Child Version that had been developed for epidemiological purposes by our group. Those scoring above 20 were interviewed for possible Obsessive Compulsive Disorder.

CT scans and PET scans of obsessive compulsive patients and age/sex-matched controls are being examined. Of particular interest are ventricle size, caudate size on the CT scans and levels of glucose utilization in the basal ganglia, cingulate gyrus, and frontal lobes.

Major Findings: Drug Treatment Trial: There was a dramatic improvement in obsessions and compulsions on CMI compared with DMI. This was striking for all measures of severity, and for global functioning as well. DMI appeared to have a mild antidepressant effect when given first, but when it followed CMI there was an increase in global depression ratings probably secondary to demoralization upon return of obsessive symptoms.

CSF Monoamines: Preliminary analysis of the first 17 CSF samples for monoamines and metabolites were carried out in the Laboratory of Clinical Science (Dr. William Potter, Chief). There was a highly significant correlation between 5-HIAA and HVA ($r=.83$) CSF 5-HIAA was negatively correlated with baseline severity; that is, the most severely ill subjects had a lower CSF 5-HIAA ($r=-.73$ $p < .01$). Unlike previous reports however, we had no relationship between baseline CSF values and drug response for these subjects.

Sydenham's chorea: Because of spontaneous outbreaks of Rheumatic fever and in some cases, Sydenham's chorea in 1986, we were able to investigate whether this infectious disorder which selectively affects the basal ganglia would be associated with obsessive compulsive symptomatology, as clinical reports from the 1940's had suggested. To date, a total of 11 adolescents with Rheumatic

fever and 11 with Sydenham's chorea have completed the Leyton Obsessional Interview. The results show that there is a significantly higher interference score regarding Sydenham's patients than by those with the Rheumatic fever. Every subject with a score of above 20 was interviewed clinically. There were two such subjects in the Sydenham's group and both had clinical OCD; none in the Rheumatic fever group had such elevated scores and so none were interviewed. This is a startling finding as our previous epidemiological data showed an incidence of less than one percent in the general population compared with 20 percent in our children with Sydenham's chorea.

Brain Imaging studies: CT scans: To date, 10 male obsessive compulsives and 10 controls have been compared with volumetric analysis of basal ganglia and other brain studies. There was a decrease in the caudate volume for the obsessive subjects compared with controls. A total of 17 PET scans have been completed in adult obsessive compulsive patients, but results are not yet available.

Significance to Mental Health Research: Childhood Obsessive Compulsive Disorder occurs in up to 1% of adolescents. There has been virtually no research on this disabling disorder, in spite of this prevalence. These studies address the etiology, treatment, and clinical course of the disorder which will have broad implications for the neurobiology of anxiety and of stereotyped behaviors.

Proposed Course of Project: A follow-up study of the 20 adolescents identified in the Columbia University epidemiological study is underway. This will show the natural course of the disorder in a community population. The drug treatment study, brain imaging studies, and surveys of subjects with Sydenham's chorea will be continued to enlarge the samples. Finally, a prospective treatment study of 30 obsessive compulsive adolescents who participated in the CMI/DMI comparison, will be initiated to see if behavioral and pharmacological treatment affect long term (two years) outcome.

Publications

Flament, M., Rapoport, J.L., Murphy, D., Berg, C., Lake, C.: Biochemical changes during clomipramine treatment of childhood obsessive compulsive disorder. Arch. Gen. Psychiatry 44: 219-225, 1987.

Berg, C., Behar, D., Zahn, T. and Rapoport, J.L.: Obsessive Compulsive Disorder-An Anxiety Syndrome? In Gittelman, R. (Ed.): Anxiety Disorders. New York, Guilford Press, 1987, pp. 126-139.

Rapoport, J.L.: Treatment of Obsessive compulsive adolescents with clomipramine and desmethylimipramine; A double-blind crossover study. Psychopharmacol. Bull., in press.

Leonard, H. and Rapoport, J.L.: Relief of obsessive compulsive symptoms by LSD and psilocin in a 17-year old obsessive compulsive boy. Am. J. Psychiatry, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00161-09 CHP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders)

Behavioral Effects of Dietary Substances in Normal and Hyperactive Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Markus Kruesi, M.D., Senior Staff Fellow, CHP, NIMH

Martine Flament, M.D., Guest Researcher, CHP, NIMH

Marian Yarrow, Ph.D., LDP, NIMH

Carolyn Zahn-Waxler, M.D., LDP, NIMH

Thomas Uhde, M.D., BPB, NIMH

COOPERATING UNITS (if any)

Laboratory of Developmental Psychology, NIMH

Biological Psychiatry Branch, NIMH

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.75

PROFESSIONAL:

.50

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Relationships between aggression, activity, sugar, and aspartame were investigated in 30 preschool boys: 18 had histories of behavioral reactions to sugar and 12 were familiar peers whose parents reported their children were not sugar reactive. Analysis of week long diet diaries found no significant differences in sugar or carbohydrate consumption between the two groups nor were correlations reported previously by another group between weekly sugar consumption and observed activity and aggression replicated. Furthermore, aggression did not increase in response in blinded sugar challenge. A significant statistical but negligible decrease in actometer activity was observed following aspartame. The likelihood of behavioral reactions to aspartame seem questionable.

Project Description:

Objectives: The relationship between dietary substances and behavior in normal and disturbed children were investigated in a series of studies; responses to sugar and aspartame were examined. Pathological responses such as aggressivity to sugar and anxiety to caffeine were tested in high risk populations.

Methods Employed: Thirty preschool boys (18 alleged sugar reactors and 12 familiar peers without such a history) received glucose (1.75 gm/kg), sucrose (1.75 gm/kg), aspartame (30 mg/kg) and a saccharin sweetened control both at home and in an NIMH playroom. The dependent measures for the NIMH sessions were videotaped observations of behavior, behavioral rating scales, and actometer measured activity. Challenges were also administered in a random order double blind manner at home. Week-long diet diaries were kept by parent(s).

Major Findings: This was the first study to investigate an etiologic role for sugar ingestion in aggression. A previous study by other investigators had reported correlations between sugar intake and aggressive-destructive, as well as hyperactive behaviors and subsequently spawned intense interest in sugar's effect upon behavior. That set of correlations was not replicated. On none of the dependent measures was an effect attributable to sugar seen. Although children reported to be reactive to sugar were more hyperactive than their peers at baseline, there was no evidence for differential reactivity to sugar.

Significance to Mental Health Research: This is the first trial to investigate an etiologic role for sugar in aggression. In addition, this study failed to replicate correlations between habitual sugar consumption and observed behavior that instigated much research in this area. Questions had arisen concerning possible adverse behavioral consequences of aspartame consumption by children either by altering central serotonin or via some unknown mechanism. This study utilized a dose estimated to be at the 90th percentile consumption for aspartame and did not find any behavioral effects. These results should direct research efforts focus upon other pathogenic influences, and reassure public concerns.

Proposed Course of Project: This project has been terminated.

Publications:

Kruesi, M.J.P. and Rapoport, J.L.: Diet and human behavior: How much do they affect each other? Annu. Rev. Nutr. 6: 113-130, 1986.

Kruesi, M.J.P.: Carbohydrate intake and children's behavior. Food. Technol. 40: 150-152, 1986.

Kruesi, M.J.P., Rapoport, J.L., Berg, C., Stables, G. and Bou, E.: 7-day carbohydrate and other nutrient intakes of preschool boys alleged to be behavior responsive to sugar intake and their peers. In Essman, W. (Ed.): Nutrition and Brain Function, Basel, S. Karger, 1987, pp. 133-137.

Zahn, T., Rapoport, J.L.: Acute autonomic nervous system effects of caffeine in boys and men. Psychopharmacology 91: 40-44, 1987.

Kruesi, M.J.P., Rapoport, J.L., Cummings, E.M., Berg, C., Ismond, D.I., Flament, M., Yarrow, M. and Zahn-Waxler, C.: Sugar and aspartame: Aggression and activity. Am. J. Psychiatry, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

701 MH 00178-06 CHP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Structure and Function in Developmental Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith M. Rumsey, Ph.D., Staff Fellow, CHP, NIMH

Susan Swedo, M.D., Staff Fellow, CHP, NIMH

Connie Duncan, Ph.D., Staff Fellow, LPP, NIMH

Richard Coppola, Ph.D., LPP, NIMH

Daniel Weinberger, M.D., Chief, CBDB, NIMH

Martha Denckla, M.D., Chief, Autism & Behav. Dis. Sec., DNB, NINCDS

Michael Goldberg, M.D., LSR, NEI

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, NIMH; Sec. on Autism & Behav. Dis., DNB, NINCDS; Clinical Brain Disorders Branch, NIMH; Lab. of Sensorimotor Research, NEI

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.1

PROFESSIONAL:

1.20

OTHER:

1.80

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Regional cerebral blood flow data, using a xenon inhalation technique, shows an increased hemispheric asymmetry and reduced antero-posterior difference in severe and persistent dyslexia. Analysis of EEG spectra, event-related potentials, and neuropsychological testing is in progress. Preliminary eye movement data has been collected on adults with poor reading and adults with attention deficit disorder, residual type (ADD-RT) to see if saccadic intrusions and related variables differentiate these groups. Tasks were designed for use in PET studies of dyslexic, ADD-RT, and autistic subjects. These include a phonological and a syntactic task designed to activate left perisylvian cortex, environmental sounds, timbre and tonal memory tasks designed to activate right temporal regions, attentional tasks designed to activate frontal cortex, and affective and attentional-shift tasks designed to tap into deficits associated with autism.

The sample of autistic men previously studied with PET and the fluorodeoxyglucose technique was increased, and a correlational pattern analysis of glucose metabolic data completed. Main findings were fewer large positive correlations between frontal and parietal regions and lower correlations of thalamus, caudate, lenticular nucleus, and insula with frontal and parietal regions in autistic patients, relative to controls.

Project Description:

Objectives: These studies have as their primary goal the identification of neuroanatomical, neurophysiological, and neuropsychological abnormalities which characterize the developmental disorders of dyslexia and infantile autism. The role of attentional dysfunction in these disorders and the relationship between dyslexia and attention deficit disorder, which show a high incidence of clinical overlap, are also of interest. A secondary goal is the determination of the sensitivity and specificity of various imaging techniques, with particular reference to developmental disorders.

Methods Employed: Methods include PET using fluorodeoxyglucose to study energy metabolism and using (0-15)-labelled water to study regional cerebral blood flow with neuropsychological activations, infrared oculography for recording eye movements, xenon inhalation techniques for measuring cortical blood flow, electrophysiology, neuropsychological testing, and behavioral questionnaires.

Major Findings: Regional cerebral blood flow studies of severely dyslexic men using xenon inhalation techniques with various activation tasks have demonstrated increased hemispheric asymmetries (including increased flow to the left hemisphere) and reduced antero-posterior gradients in these men, relative to controls. The former suggests that there may be less efficient information processing or inadequate bihemispheric integration, rather than the decreased ability to activate left language cortex that has been hypothesized to be involved in dyslexia. The reduced antero-posterior difference may reflect a deficit in the ability of frontal systems to respond adequately to cognitive demands. This finding parallels the reduced antero-posterior differences in glucose metabolism seen in adults with ADD-RT reported by Zametkin, et al (1986). Taken together, the two studies suggest the possibility of some common substrate for these two frequently overlapping disorders.

Neuropsychological studies indicate that deficits in verbal learning associated with severe dyslexia involve not only immediate memory span, but also deficits in application of semantic strategies, which normally facilitate recall.

Correlational analyses of PET-FDG data on 14 autistic men and their controls show fewer large positive correlations between frontal and parietal regions and lower correlations of the thalamus, caudate, lenticular nucleus, and insula with frontal and parietal regions in autistic patients, with many of the latter correlations negative in the autistic group that are positive in controls. These results may indicate functionally impaired interactions between frontal/parietal regions and neostriatum and thalamus, regions which subserve directed attention.

Eye movement data has been collected using infrared oculography in small numbers of subjective poor readers (i.e. individuals who complain of slow reading, but who do well on standardized tests), and adults with attention deficit disorder, residual type and in 15 normal controls. Additional data collection and quantification of existing data is underway to determine the incidence and nature of saccadic intrusions in these groups. Regressions while reading appear to distinguish subjective poor readers from the other groups. Thus, despite their good performance on standardized reading measures, eye movement data document the legitimacy of their reading complaints.

Significance to Mental Health Research: Application of these techniques holds promise for understanding developmental disorders, the role attentional dysfunction plays in them, and their relationship to other child psychiatric disorders, such as attention deficit disorder. This research may prove useful for understanding brain dysfunction in disorders where macroscopic brain anatomy appears normal. Such dysfunction may involve widely distributed neural systems, rather than mimicking the more circumscribed localization seen in acquired disorders involving focal lesions. These studies may also aid in the development of a more meaningful and biologically-based nosology. The eye movement data may highlight a large and previously understudied group of "subjective poor readers". This work will differentiate them from dyslexic subjects and possibly suggest new treatment strategies.

Proposed Course of Project: A protocol for further study of regional cerebral blood flow using neuropsychological activations in dyslexia, as well as in ADD-RT and infantile autism, residual state, will be activated with PET. Nine tasks have been developed for pretesting and are being audiotaped. These tasks will be pretested behaviorally in normal controls and in small samples of the various patient groups for their sensitivity to processing deficits and will be pretested physiologically with PET for the ability to differentially activate brain regions of interest in these disorders. In dyslexia and ADD-RT, we are interested in activating left anterior and posterior language cortex and prefrontal cortex with attentional, phonological and/or syntactic tasks. Tasks involving environmental sounds, tonal memory, and timbre will be used to activate right temporal cortex as a contrast region. These two disorders will be deconfounded in this study. Because autistic patients frequently show expressionless faces and flat intonation, tasks involving the perception and expression of affective intonation are also being pretested. A task involving the shifting of attention will also be pretested in this group.

Once task selection is completed, we will proceed with our PET study of dyslexia and attention deficit disorder, while completing additional behavioral studies of autism in adults with relatively high Verbal and Performance IQs. The latter will provide important additional information for task selection for PET studies of autistic men.

A correlational pattern analysis is being applied to our cerebral blood flow data on severe dyslexia. Of particular interest, given our findings of unusually large hemispheric asymmetries and reduced antero-posterior differences, are correlations between left-right homologous cortical regions and frontal-parietal association cortex. Group differences in correlational patterns involving these areas would suggest unusual functional associations between these sets of regions.

Analysis of preliminary eye movement data and of electrophysiological data is planned for this year. Results will determine our decisions concerning future application of these methods.

We are also pursuing comparative neuropsychological studies of men with mild and with "compensated" versus severe dyslexia and of dyslexic men with and without attention deficit disorder. Such studies will provide a meaningful context within which to evaluate the generality of our physiological findings.

Publications

Rumsey, J., Dorwart, R., Vermess, M., Denckla, M.B., Kruesi, M.J. and Rapoport, J.L.: Brief report: Magnetic resonance imaging of brain anatomy in severe developmental dyslexia. Arch. Neurol. 43(10): 1045-1046, 1986.

Rumsey, J., Andreasen, N. and Rapoport, J.L.: Thought, language, communication, and affective flattening in autistic adults. Arch. Gen. Psychiatry 43: 771-777, 1986.

Creasey, H., Rumsey, J., Schwartz, M., Duara, R., Rapoport, J.L. and Rapoport, S.: Brain morphometry, as measured by quantitative CT scanning, in autistic men. Arch. Neurol. 43(7): 669-672, 1986.

Zahn, T., Rumsey, J. and Van Kammen, D.P.: Autonomic nervous system activity in autistic, schizophrenic, and normal men: Effects of stimulus significance. J. Abnorm. Psychol. 96(2): 135-144, 1987.

Rumsey, J. and Denckla, M.: Neurobiological research priorities in autism. In Schopler, E. and Mesibov, G.(Eds.): Current Issues in Autism. New York, Plenum Publishing Corp., 1987, pp. 43-61.

Rumsey, J., Creasey, H., Stepanek, J., Dorwart, R., Patronas, N., Hamburger, S. and Duara, R.: Hemispheric asymmetries, fourth ventricular size, and cerebellar morphology in autism. J. Autism Dev. Disord., in press.

Rumsey, J., Berman, K., Denckla, M., Hamburger, S., Kruesi, M. and Weinberger, D.: Regional cerebral blood flow in severe developmental dyslexia. Arch. Neurol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00301-05 CHP

PERIOD COVERED

October 1, 1986 to September 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Diagnosis in Child Psychiatry

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith L. Rapoport, M.D., Chief, CHP, NIMH
Breck Borcherding, M.D., Clinical Associate, CHP, NIMH
Alan J. Zametkin, M.D., Staff Psychiatrist, CHP, NIMH
Eric Taylor, M.D., Sr. Registrar, Maudsley Hospital, London, Eng.
James Swanson, Ph.D., Prof. of Psychology, Univ. of Calif., Irvine, CA
Michael Rutter, M.D., Prof. of Child Psychiatry, Maudsley Hospital, London, Eng.

COOPERATING UNITS (if any)

Department of Psychiatry, Maudsley Hospital, London, Eng.
Department of Pediatrics, University of California, Irvine, CA

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.30

PROFESSIONAL:

.20

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To investigate the basis for widely separate rates in the diagnosis of childhood hyperactivity between the U.S. and U.K., research teams and clinician panels in both countries completed diagnostic ratings of standardized case histories using DSM III and ICD 9 diagnostic schemes. The results showed effects of diagnostic schemes on rates for the diagnosis of hyperactivity.

However, there was additional effect of nationality of case, with U.S. cases more likely to receive the diagnosis of Attention Deficit Disorder. To pursue this, a new study is started comparing 24 hour motor activity in hyperactive children and their parents in three settings: Bethesda, Maryland; Irvine, California; and London, England.

Project Description:

Objectives: To examine the widely discrepant rates of diagnosis of childhood hyperactivity between the U.S. and most European countries, hyperactive boys are being studied with 24-hour actometer recordings of naturalistic motor activity in three different clinical settings. The purpose of the study is to see whether, at a given score on teacher and parent rating scales, children actually exhibit comparable motor activity across these settings.

Methods Employed: Approximately 15 boys, ages six to 11 years, will be studied at each setting - the Child Psychiatry Branch at NIMH, the Department of Pediatrics at the University of California at Irvine, and the Division of Child and Adolescent Psychiatry at the Maudsley Hospital in London, England. Boys chosen will meet DSM III-R criteria for Attention Deficit/Hyperactivity Disorder and have comparable and appropriately high parent and teacher ratings of disruptive behavior.

These children will wear activity monitors for seven consecutive days of 24-hour recording of activity levels. The results will be compared across U.S. and European settings. Specific comparisons of activity during different time periods, such as sleep, play, and school subject, will also be made.

Major Findings: None to date from the actometer study. A major effect of clinician training and diagnostic scheme has been found.

Significance to Mental Health Research: Childhood hyperkinesis has been considered an American phenomenon and widely ascribed to dietary, cultural and/or environmental as has been supposed and that most of the differences is accounted for by clinician rating scheme and possibly referral differences in the two countries.

Data from this project may confirm that actual activity levels are comparable in both the English and American boys, when a similar diagnostic scheme is used.

Proposed Course of Project: The above data will be collected throughout the school year and provide a basis for further study of combinations of ratings, activity level, and cognitive testing to compare diagnosis across different settings.

Publications:

Rapoport, J.L., Donnelly, M. and Zametkin, A.: Situational hyperactivity in a United States clinical setting. J. Child Psychol. Psychiatry 27: 639-646, 1986.

Taylor, E. and Rapoport, J.L.: Diagnosis of Hyperactivity - U.S. & UK Differences. In Sargeant, J. and Bloomingdale, L. (Eds.): Research Diagnostic Criteria for Attention Deficit Disorder. New York, Spectrum Publishers, in press.

Rapoport, J.L.: DSM III-R and Child Diagnosis. In Last, C. and Hersen, M. (Eds.): Issues in Diagnostic Research. New York, Academic Press, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02240-03 CHP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Neurobiology of Attention Deficit Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith L. Rapoport, M.D., Chief, CHP, NIMH

Alan J. Zametkin, M.D., Staff Psychiatrist, CHP, NIMH

Markus J.P. Kruesi, M.D., Staff Psychiatrist, CHP, NIMH

William Z. Potter, M.D., Ph.D., Chief, Section on Clinical Pharmacology, LCS,
NIMH

Markku Linnoila, M.D., Ph.D., Chief, LCS, NIAAA

Robert M. Cohen, M.D., Ph.D., Chief, Section on Clinical Brain Imaging, LCM, NIMH

COOPERATING UNITS (if any)

Section on Clinical Pharmacology, LCS, NIMH

National Institute on Alcohol Abuse and Alcoholism

Section on Clinical Brain Imaging, LCM, NIMH

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.75

PROFESSIONAL:

2.0

OTHER:

.75

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To localize possible abnormal patterns of cerebral glucose metabolism and cerebral blood flow in adults with Attention Deficit Disorder (ADD) Residual Type, parents of diagnosed hyperactive children are being studied using the 2-deoxyfluoroglucose method of Positron Emission Tomography (PET).

To date, 30 PET scans have been performed without medication and 16 scans have been done during treatment with dextroamphetamine. To understand the mechanism of action of stimulant medication in the treatment of Attention Deficit Disorder with Hyperactivity (ADHD), a comparison of pemoline (Cylert), methylphenidate, and dextroamphetamine was initiated to examine the acute and chronic effects of these medications in the same subjects on peripheral measures of dopamine, norepinephrine, prolactin, and growth hormone.

The relationships between the behavioral dimensions of impulsivity, aggression, and concentrations of monoamine metabolites, particularly 5-HIAA, in the spinal fluid of children with conduct disorder and/or attention deficit disorder, is being examined. Associations between decreased CSF 5-HIAA concentrations and impulsive aggressive behavior have been reported in impulsive adults. Peripheral measures of serotonin and catecholamines are being obtained along with personality, impulsivity and aggression measures. Seventeen subjects have been studied to date. A preliminary comparison between ten Conduct Disorder and ten age-matched Obsessive Compulsive children, shows a trend ($p = .09$) for lower CSF 5-HIAA concentration in conduct disorder. Within the conduct disorder sample, lower values are associated with more destructive behavior.

Project Description:

Objectives: To study the pathophysiology of ADDH and Conduct Disorder. Specific monoamine and neuroanatomical hypotheses are being tested using pharmacological probes, examination of spinal fluid, blood and urinary excretion of monoamines and metabolites, and with Positron Emissions Tomography (PET). Understanding biological contributors to these disorders will aid in development of more effective treatment.

Methods Employed: In earlier studies we have demonstrated that methylphenidate, dextroamphetamine, and pemoline have different effects on peripheral measures of catecholamine function. Current work examines whether in the same sample of children, all three drugs act differently by studying children during a multiple stimulant drug crossover study. Biochemical correlates of response will be examined.

Spinal fluid monoamine metabolites are being obtained from children with conduct disorder, attention deficit disorder, children with obsessive compulsive disorder as well as a pediatric contrast group. Platelet serotonin, as well as plasma catecholamines, are being collected from the psychiatric subjects. Measures include ratings of impulsivity, aggression, anxiety, depression, and obsessiveness.

Major Findings: Pemoline appears as clinically effective as methylphenidate and dextroamphetamine. When children are examined on the day hospital setting, there are very few non-responders to any of the three stimulant medications. Isolated cases of selective response and of tolerance to stimulant drug response are of considerable theoretical interest and are being studied in greater depth.

To document the presence in children of a concentration gradient for dopamine and serotonin metabolites in the CSF, (which is well established in adults), sequential CSF aliquots were obtained from eight prepubertal subjects and 18 subjects who were Tanner stage II to V. Three of eight prepubertal subjects had slopes for 5-HIAA concentration/ml collected that were not statistically different from zero. In contrast, across the whole, group slopes were similar to those reported in adults. Comparison of ten age-matched conduct disorder and obsessive compulsive disorder children showed a trend ($p = .09$) for lower concentrations of 5-HIAA in the conduct disordered group. Within the conduct disorder group ($N=13$) there is a significant negative correlation ($r=-.63$) between CSF 5-HIAA and Brown-Goodwin Scale ratings of aggressive acts.

The PET studies are not yet analyzed.

Significance to Mental Health Research: Attention Deficit Disorder is a relatively common childhood disability that persists into adulthood for about 30% of the cases. Examination of pathophysiology of childhood ADDH and Conduct Disorder will permit more accurate diagnosis and treatment of children and adults with a spectrum of impulse disorders.

Conduct disorder is the most frequent cause for referral of children and adolescents for psychiatric services. The preliminary trend for lower 5-HIAA concentration in the CSF of conduct disorder subjects is consistent with studies in adults relating impulsive and aggressive behavior with decreased 5-HIAA concentrations. Identification in children of a concentration gradient for the monoamine metabolites, 5-HIAA and HVA, establishes the need for methodological control over the aliquot collected - a point not addressed in previously in pediatric behavioral studies.

Proposed Course of Project: The sample size of the pemoline study will be increased to approximately 20 and the data will then analyzed. PET scans will continue to enlarge the sample size of drug treated subjects both on and off stimulant medication.

The data on CSF concentration gradients and the contrasts between pre and post pubertal children is being analyzed, as are preliminary examination of dimensions of personality variables and aggression histories across subjects in relation to CSF biochemistry.

A follow-up study is underway of all subjects for whom CSF chemistries are available to see if 5-HIAA and/or HVA predict follow-up status.

Publications:

Kruesi, M.J.P., Linnoila, M., Rapoport, J.L., Brown, G.C. and Petersen, R.: Carbohydrate craving, conduct disorder and low CSF 5-HIAA. Psychiatry Res. 16: 83-86, 1985.

Zametkin, A.J., Linnoila, M., Karoum, F. and Salle, R.: Pemoline and the urinary excretion of catecholamines and indolamines in children with attention deficit disorder. Am. J. Psychiatry 143:3: 359-362, 1986.

Donnelly, M., Zametkin, A.J., Rapoport, J.L., Ismond, D., Weingarten, H., Lane, E., Oliver, J., Linnoila, M. and Potter, W.Z.: Treatment of childhood hyperactivity with desipramine: Plasma drug concentration, cardiovascular effects, plasma and urinary catecholamines and clinical response. Clin. Pharmacol. Ther. 39:1: 72-81, 1986.

Rapoport, J.L., Donnelly, M., Zametkin, A.J. and Carrougner, J.: Situational hyperactivity in a U.S. clinical setting. J. Child Psychol. Psychiatry 27:5: 639-646, 1986.

Zametkin, A.J. and Rapoport, J.L.: The Pathophysiology of Attention Deficit Disorder. A Critical Review. In Lahey, B. and Kasdin, A. (Eds.): Advances in Clinical Child Psychology. New York, Plenum, 1986, pp. 177-216.

Zametkin, A.J., Rapoport, J.L., Potter, W.Z. and Karoum, F.: Treatment of hyperactive children with d-phenylalanine. Am. J. Psychiatry 144: 792-793, 1987.

Zametkin, A.J. and Rapoport, J.L.: The Noradrenergic Hypothesis of Attention Deficit Disorder: A Critique. In Meltzer, H. (Ed.): Psychopharmacology: Third Generation of Progress. New York, Raven Press, 1987.

Zametkin, A.J. and Rapoport, J.: The neurobiology of attention deficit disorder with hyperactivity. Where have we come in 50 years? J. Am. Acad. Child Adolesc. Psychiatry, in press.

Zametkin, A.J., Potter, W.Z. and Rapoport, J.L.: The effect of methylphenidate upon urinary catecholamines and behavior in hyperactive children: A replication. Biol. Psychiatry, in press.

Prendergast, M., Taylor, E., Rapoport, J.L., Zametkin, A.J., Donnelly, M., Aist, M.B. and Bartko, J.: a cross-national study of DSM-III and ICD-9 psychiatric diagnosis of children with disorders of conduct and/or attention. J. Child Psychol. Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00084-13 CNG

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic-Biologic Studies of Psychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I.	E. Gershon	Chief	CNG, NIMH
Others:	W.H. Berrettini	Medical Officer	CNG, NIMH
	L. DeLisi	Asst. Professor	SUNY at Stony Brook
	J.I. Nurnberger Jr.	Dir. of Res., Inst. of Psych. Res., Indiana U.	
	J. Hamovit	Research Social Worker	CNG, NIMH
	J. Guroff	Social Science Analyst	CNG, NIMH
	D. Kazuba	Psychologist	CNG, NIMH

COOPERATING UNITS (if any)

LCS, NSB, NIMH, Chestnut Lodge, Rockville, MD

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Section on Clinical Genetics

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20892

TOTAL MAN-YEARS:

8.8

PROFESSIONAL:

4.5

OTHER:

4.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Family study of schizophrenia: 108 relatives of chronic schizophrenics, 129 relatives of chronic psychosis schizoaffectives, and 380 relatives of psychiatrically normal controls, were studied with systematic diagnostic interviews, information from relatives, and review of medical records where appropriate. Comparing relatives of schizophrenics with relatives of schizo-affectives, there was no tendency for schizoaffective diagnosis or acute or chronic psychoses to aggregate separately. Increased bipolar disorder was found in relatives of schizoaffectives but not in relatives of schizophrenics. Major affective disorder (bipolar and unipolar) was increased in relatives of both types of psychotic proband. A separate family study of bulimia is ongoing.

Cultured lymphoblastoid cell lines have been prepared from a series of 40 affected-sib-groups with schizophrenia, 16 pedigrees of bipolars, and 5 schizophrenia pedigrees. These are being used in genetic linkage and association studies.

Mathematical analysis of the power of linkage methods has provided estimates of the robustness, in the presence of genetic heterogeneity, of the affected-sib-pair and pedigree lod score linkage methods (study of unaffected offspring) for inherited quantitative traits. We have also analyzed the power of the high-risk paradigm. These analyses allow precise estimates of the required numbers of individuals for valid linkage and high risk studies.

Investigators (continued)

I.D. Dauphinais	NRSA Fellow	CNG, NIMH
J. Gelernter	Medical Staff Fellow	CNG, NIMH
J. Gejman	Visiting Associate	CNG, NIMH

I. Family studies

A. Schizophrenia

The initial report of this study, a collaboration with the Clinical Neuroscience Branch and Chestnut Lodge, has been accepted for publication.

237 relatives of 48 patients with chronic psychosis, diagnosed as either schizophrenia or schizoaffective disorder, along with 380 relatives of psychiatrically normal controls, were studied with systematic diagnostic interviews, information from relatives, and review of medical records where appropriate. A variety of nonbipolar psychotic disorders was found in the relatives of the patients. Comparing relatives of schizophrenics with relatives of schizoaffectives, there was no tendency for schizoaffective diagnosis or acute psychoses to aggregate separately from schizophrenia. Increased bipolar disorder was found in relatives of schizoaffectives but not relatives of schizophrenics. Major affective disorder (bipolar and unipolar) was increased in relatives of both types of psychotic probands (see table next page). If we subdivide the ill probands according to whether or not they had a history of substance abuse, relatives of probands with substance abuse had greater frequency of affective disorder and of substance abuse, but there were not significant differences in number of relatives with non-bipolar psychoses.

These results have important implications for molecular genetic linkage studies of schizophrenia. In large pedigrees, one can lose considerable statistical power by failing to identify as "affected" relatives who have variant forms of illness. This study supports an inclusive definition of "affected" -- schizoaffective, drug abuse with chronic psychosis, and possibly major affective disorder, in these families. Of course, the schizotypal personality disorders previously associated with schizophrenia would also be included. Another implication of these family study results is to look for common linkage markers in schizophrenia and schizoaffective disorder.

Illness Frequencies in First-Degree Relatives

Age-Corrected Morbid Risk, per cent

<u>Proband Diagnosis</u>	<u>All Psychosis*</u>	<u>Schizo- Affective Acute</u>	<u>All Bi- polar**</u>	<u>Unipolar</u>	<u>Other & Normal</u>	<u>Number of Relatives</u>
Schizophrenia Chronic (24 families)	6.2	5.0	2.2	14.7	71.9	108
Schizo- Affective Chronic (24 families)	11.7	0.0	9.6	9.3	69.4	129
Control (67 families)	2.1 ¹	0.3 ²	0.8 ³	6.7 ^{4,5}	90.1	380

* Includes schizophrenia, schizoaffective chronic and indeterminate, unspecified functional psychosis, other psychiatric disorder with hospitalization.

**Includes bipolar I, bipolar II, cyclothymic personality.

¹ p = 0.03 vs schizophrenia, p < 0.0001 vs schizoaffective

² p < 0.01 vs schizophrenia

³ p < 0.0001 vs schizophrenia

⁴ p = 0.01 vs schizophrenia

⁵ All affective (all bipolar and unipolar): p = 0.007 vs schizophrenia, p < 0.001 vs schizoaffective

All probabilities by Fisher's exact test.

B. Bulimia

In collaboration with Dr. Jimerson of Laboratory of Clinical Science, a family study of bulimia is ongoing. 42 probands have been enrolled in the study, and 67 relatives of patients have been examined. The controls are the same as in the schizophrenia family studies. We propose to determine whether affective disorders and eating disorders are co-aggregating in families of these patients. The study should be complete within 18 months.

III. Population genetic studies

1. Power of linkage methods

A. Affected sib-pair method

In preliminary studies, we determined the number of affected-sib-pairs needed to find linkage for some specific modes of inheritance and for a small amount of heterogeneity. We have extended these studies and examined the required sample size for a wide range of assumptions that are likely to be relevant for the major psychiatric disorders. We have considered the merits of applying this methodology for screening the human genome for linkage to psychiatric disorders as well as replicating linkages found in specific pedigrees in a more general population sample. For example, to find linkage to affective disorders (assuming 7% population prevalence and autosomal dominant inheritance with reduced penetrance), as many as 160 affected sib-pairs would be needed to find linkage up to 10% recombination if only 50% of families were linked to the marker locus. To determine if the chromosome 11 linkage found in an Amish pedigree could be replicated in a sample of affected-sib-pairs, only about 1/3 of this sample size would be required because the linkage is close. About the same number would be required to replicate this finding of chromosome 21 linkage to Alzheimer's disease. However, if only a small proportion of families were linked (25%), then 220 affected-sib-pairs would be needed. In general, the affected-sib-pair method will be more powerful for "rarer" disorders like schizophrenia than for "common" disorders like major affective disorder. In any case, a new linkage will be difficult to detect unless it accounts for about 50% of families. However, this method will usually be powerful for determining whether a linkage previously found in a limited sample for sub-population is present in the general population, and for estimating the proportion of families in the population that are linked.

B. Lod score method

We have examined the power of the lod score method for pedigrees of "moderate" size (approximately 15 individuals) under conditions of heterogeneity. For example, a highly penetrant, dominant gene for bipolar illness (given the parameters found to fit linkage in the Amish pedigree) that accounted for 25% of families, about 30 families of this size would be needed to insure a high probability to detect linkage. However, if only 10% of families were linked, we would only have 50% chance to detect linkage with 30 families. Thus, a substantial number of families are required to find linkage if this gene accounts for only a small proportion of families. If penetrance of the gene were reduced (50%), the overall power is slightly lower. These sample size estimates are conservative since, given the rapid progress in the development of the human gene map, most linkage studies will examine multiple marker loci simultaneously (whose map positions are known) which increases the power of linkage detection over pairwise tests.

2. Power of high risk methods for biological traits

We originally examined the power of studying high risk samples (offspring of one or more affected parents who are at risk for developing illness) in order to confirm that potential biological trait markers are not a consequence of the ill state. We originally calculated the power of this method for a simple normally distributed trait. We have extended these studies to consider how powerful the high risk method is when a trait is dichotomous or when there are large differences in the variance of a quantitative trait between ill and well individuals. We have shown how these determinations depend on underlying assumptions about the mode of inheritance of the trait and its relationship to illness susceptibility. For example, using the genetic model hypothesized by Matthyse et al. to account for the familial association between schizophrenia and deviant eye tracking, we calculated that this association could be confirmed in a high risk study if about 50 individuals/group were studied (i.e., 50 offspring of one affected parent and 50 controls). To examine the effect of different variances in patients and controls, we simulated high risk samples based on the published findings of melatonin suppression in response to light in patients with affective disorders and controls. That is, we assumed that the high risk population was a mixture of individuals having different susceptibilities. Under these assumptions, we determined that 25-30 individuals/group are needed to confirm the presence of the abnormality in a high risk sample. For any trait, studying offspring of two affected individuals is substantially more powerful than studying offspring of one affected individual.

Significance to Biomedical Research and the Program of the Institute

For the long-term goal of identifying genes that cause psychiatric disorders, our present results are important milestones. The family study of schizophrenia provides definitions of affected and unaffected to be used in genetic linkage studies and inherited risk factor studies. The cultured cell lines provide the biological substrate for the linkage studies with recombinant DNA and protein markers. The mathematical analyses have defined the most powerful sampling methods to use to test linkage and candidate gene hypotheses, and provided an impetus to the study of inherited risk factors in well relatives of patients.

Proposed Course of Project

We plan to continue to investigate the biology and genetics of characteristics that may be implicated in the genetics of affective disorders, as described above. The molecular genetics approach of interindividual differences in neuropeptides and other substance will be pursued. Establishing a library of DNA and living cells from entire pedigrees is a major priority. Study of relatives at risk for affective disorders and schizophrenia will proceed. Mathematical methodology for clinical investigation will continue to be studied.

Several projects have been spun off from this one: Z01 MH 02236-03 CNG, Schizophrenia Studies, Z01 MH 02237-03 CNG, Molecular Genetics of Neuropsychiatric Disorders and Z01 MH 00086-11 CNG, Outpatient Clinic for Genetic and Pharmacological Studies of Affective Disorders, Z01 MH 0085-11 CNG, Pharmacogenetics of Psychoactive Drugs. This project will be continued as the main biologic-genetic project of the Section, and the start-up of new initiatives will be within this project.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00086 11-CNG

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Outpatient Clinic for Genetic and Pharmacologic Studies of Affective Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W.H. Berrettini	Medical Officer	CNG, NIMH
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	J.R. Hamovit	Research Social Worker	CNG, NIMH
	M.E. Maxwell	Research Social Worker	CNG, NIMH

COOPERATING UNITS (if any)

CC, NIH: CHP, BPB, LPP, LCS, NPB, LCS, NIMH: Catholic University: NSB, NIAA:
University of Pittsburgh: Karolinska Institute: LSN, NIA: Indiana University

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Section on Clinical Genetics

INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:

3.8

PROFESSIONAL:

1.5

OTHER:

2.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The high-risk study of healthy adolescent offspring of bipolar parents has identified a potential vulnerability marker in the at-risk population. The high-risk group shows supersensitivity to the inhibitory effects of light on plasma melatonin, suggesting that this finding may represent a genetic vulnerability marker for affective disorder.

We have completed a study of cholinergic induction of rapid eye movement (REM) sleep in euthymic unmedicated bipolars. The bipolars showed more rapid induction of REM sleep than did matched controls, confirming previous observations and providing additional evidence that cholinergic supersensitivity may be a genetic vulnerability marker for affective disorder.

Studies of the growth hormone response to clonidine in euthymic unmedicated bipolars suggest that this response is blunted in bipolar subjects compared to controls. The possibility that this represents a genetic vulnerability marker is currently being evaluated.

Studies of CSF neuropeptides in normal monozygotic and dizygotic twins have shown that neuropeptide Y levels are heritable, are high in underweight anorectics and are normal in depressives. Additionally, the related neuropeptide, peptide YY, is elevated in CSF from bulimic subjects. Galanin levels in CSF are normal in Alzheimer's Disease, depression and eating disorders.

Acute IV administration of physostigmine produces increases in CSF neuropeptide Y, in both monkeys and man, indicating that pharmacologic challenges may employ CSF neuropeptide levels in assessing the drug effect on various CNS neurotransmitters.

Investigators (Continued):

J. Gelernter	Medical Staff Fellow	CNG, NIMH
L. Goldin	Senior Staff Fellow	CNG, NIMH
L. Sapin	Guest Researcher	CNG, NIMH
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D. Pellegrini	Asst. Prof. of Psychology	Catholic Univ.
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M. Linnoila	Clinical Director	NIAAA
P. Gold	Section Chief	BPB, NIMH
C. May	Senior Staff Fellow	LNS, NIA
L. Tamarin	Clinical Associate	CPB, NIMH
D. Rubinow	Unit Chief	BPB, NIMH
G. Oxenstierna	Staff Psychiatrist	Karolinska Inst.
G. Sedvall	Professor of Psychiatry	Karolinska Inst.

Project Description

We maintain an outpatient clinic of approximately 100 bipolar subjects for the purpose of: 1) identifying potential markers of genetic vulnerability to affective disorder; and 2) studying the onset and course of illness of bipolar disease. In addition to the bipolar subjects themselves, we are conducting a study of the healthy adolescent children of bipolar parents, children at "high risk" for the development of affective disorder.

We study euthymic (well-state) bipolar subjects to determine those abnormalities which are most likely to be inherited characteristics of the illness. Additionally, the study of children at high risk for affective illness is useful in avoiding drug effects or secondary effects of the illness which may persist even in the euthymic state.

For comparison purposes we maintain a pool of approximately 50 well-screened normal volunteers.

Lastly, this project includes an active laboratory devoted to the study of CSF neuropeptides in psychiatric diseases. This laboratory has collaborated with clinical investigators, attempting to elucidate the roles of various neuropeptides in eating disorders, affective illness, Alzheimer's disease and schizophrenia.

Each aspect of the project is described below.

1. HIGH RISK STUDY

We are continuing a prospective study of the healthy adolescent (age 15-25) children (N=61) of bipolar parents and age-matched children (N=44) of control parents. These high-risk adolescents are being studied prior to onset of affective illness, and it is expected that 30% of them will eventually become ill. By conducting this prospective study, we hope to identify biological or psychological variables that will predict who will become ill. Subjects are interviewed annually for evidence of affective disorder. To date six children of bipolar parents have become ill (1 bipolar and 5 unipolar cases), while one control child has developed affective disorder during the first three years of the study.

One potential vulnerability marker has been identified. Unmedicated bipolar subjects, independent of mood state, show supersensitivity to the inhibitory effects of light on plasma melatonin. To evaluate this potential marker, we studied the high risk population and the control group using this paradigm in collaboration with Dr. Larry Tamarin. Ten of 23 at risk subjects were supersensitive, compared to three of 22 controls. This results suggests that the supersensitivity to light may be a genetic vulnerability marker for affective disorder. However, studies on the heritability of this response and segregation studies in pedigrees are necessary to confirm this putative marker.

2. CHOLINERGIC REM INDUCTION

We have confirmed our previous observation that euthymic unmedicated bipolars have more rapid induction of REM sleep by the cholinergic agonist, arecoline. While the difference between the bipolar and control groups was not as great as that observed in a previous study, it remained statistically significant ($p < .05$, $N=18$ in each group). These results suggest that cholinergic supersensitivity may be a genetic vulnerability marker in affective illness. However, due to the great overlap between patients and controls, this particular paradigm is not useful for diagnosis, but may be appropriate for segregation studies.

3. CSF NEUROPEPTIDES

Disease-related studies:

We have continued to study CSF neuropeptides in psychiatric disorders, to determine the extent to which CSF levels of a neuropeptide can reflect abnormal behaviors or reveal aspect of pathophysiology. For example, we have developed two new assays for measurement of galanin and peptide YY in CSF. These substances are potent stimulators of feeding behavior in rats, and so we measured their CSF levels in anorexia nervosa and bulimia (Dr. Walter Kaye). While galanin did not differentiate eating disorder subjects from controls, CSF peptide YY levels were elevated in bulimic subjects who had abstained from bingeing and purging behavior for 30 days, compared to anorectics or controls.

A third potent stimulator of feeding behavior in rats is neuropeptide Y (NPY) which was also measured in CSF from eating disorder patients. NPY was elevated in the underweight anorectics and returned to normal with weight recovery. CSF NPY levels in bulimics were not different from those of controls.

Galanin is found within 40% of the cholinergic neurons of the basal forebrain (the cells which degenerate in Alzheimer's disease). We studied CSF galanin levels in subjects with Alzheimer's disease and age-matched controls and elderly depressives (Drs. Trey Sunderland and Conrad May). No group differences were found.

Heritability studies:

To determine whether CSF neuropeptide levels are heritable we studied CSF NPY, CRF and GHRF in monozygotic and dizygotic twins and in brothers (Drs. Gabriella Oxienstierna and Goran Sedvall). NPY levels were determined by additive genetic factors while CRF and GHRF levels were influenced more by environmental factors, as shown in the table below.

Heritability of CSF Neuropeptides

	MZ TWINS (N=16)	DZ TWINS (N=12)	BROTHERS (N=11)
NPY ⁼⁼	0.59*	0.26	0.18
CRF	0.40	0.59	0.13
GHRF	0.27	0.31	0.11

⁼⁼Jensen's Heritability Index=0.66

*intraclass correlation coefficient

CSF pharmacologic challenges:

While basal CSF levels of a neuropeptide may be unchanged in a certain disease state, if a methodology could be developed to study stimulated release of a peptide from brain into CSF, abnormalities undetectable in the basal state could become apparent. We have attempted to develop such a methodology, by using IV physostigmine (up to 15 ug/kg) to provoke release of neuropeptides into CSF. Monkey experiments (Drs. Nancy Garrick and Dennis Murphy) and human studies have indicated that CSF NPY increases after 15 ug/kg IV physostigmine and that this increase can be blocked by atropine. Neostigmine is without effect. No changes were seen for somatostatin (SRIF), CRF, GHRF, vasopressin, beta-lipotropin (LPH) or vasoactive intestinal peptide (VIP). Smaller doses of physostigmine are not effective in increasing CSF NPY.

CSF Correlations:

We have observed a set of highly significant positive correlations between concentrations of unrelated neuropeptides in CSF (as shown below). The origin of these correlations is obscure, but factor analysis suggests that a single factor is responsible for 50% of the variance observed for each peptide. This observation is intriguing and deserves further study.

Correlations among CSF Neuropeptides				
	LPH	CRF	SRIF	ACTH
VIP	.69*	.57	.68	.73
	.003+	.02	.003	.001
LPH		.66	.63	.62
		.006	.008	.008
CRF			.53	.71
			.03	.001
SRIF				.59
				.01
*correlation coefficient				
+significance level				
N=16				

§. GROWTH HORMONE RESPONSE TO CLONIDINE

The growth hormone response to clonidine has been reported to be blunted in subjects with affective disorder. To determine whether this is a state-independent phenomenon, we are studying unmedicated euthymic bipolars and controls. To date 12 bipolars and 15 controls have been studied. The bipolars do show a significantly blunted growth hormone response ($p < .04$). We are currently attempting to study the same patients during lithium treatment to assess the effect of lithium on this putative marker. This measure may represent a clinically available test of alpha adrenergic function in affective disorder. Moreover, this test may be a useful method of selecting pedigrees to search for linkage to restriction fragment length polymorphisms of the alpha adrenergic receptor gene, a project which will begin shortly.

Significance to Biomedical Research and the Program of the Institute

The high-risk study has identified a putative vulnerability marker for affective illness. As a result of the annual follow-up, we will be able to determine the effect of psychosocial and biological variables on the

development of affective disorder. Additionally, through annual follow-up, we may be able to identify, early in the course of illness, subjects with affective disorder, and we may be able to delineate carefully subclinical antecedents.

The finding of elevated peptide YY in abstinent bulimics is intriguing, and is congruous with the hypothesis that bulimics binge to correct a central peptide YY abnormality, as their CSF levels are normal when they are actively bingeing. In light of the potent stimulation of feeding behavior in rats exerted by peptide YY, this finding may prove to be of etiological significance in bulimia.

The fact that an acute IV dose of physostigmine can provoke release of NPY into CSF suggests that pharmacologic challenges can employ CSF neuropeptide measurements as endpoints in assessing brain neurotransmitter systems. This methodology may be applicable to numerous drugs and different CSF neuropeptides. Measurement of a CSF endpoint may be a more useful strategy in pharmacologic challenges, which have traditionally relied on plasma, behavioral or cardiovascular measurements.

The observation that many unrelated CSF neuropeptide levels are highly positively correlated with one another indicates that a major determinant of CSF concentrations of various neuropeptides remains to be discovered. This determinant may be related to the rate of formation or absorption of CSF or the rate at which neuropeptides disappear from CSF. Because multiple studies of CSF neuropeptides are being conducted in neuropsychiatric disease, delineation of the determinant(s) would be a major contribution to our understanding of the significance of CSF neuropeptide concentrations.

The clonidine studies confirm previous reports of blunted growth hormone responses in affective disorder. The persistence of this finding in the euthymic state suggests that this may be a state-independent marker for affective illness.

Proposed Course of Study

The high-risk study will continue as planned with annual follow-up of the high-risk and control groups. It is anticipated that additional subjects will be studied for melatonin inhibition induced by light. In addition, we will begin studies of bipolar subjects using Psoralen, an agent which provokes release of melatonin from the pineal gland.

We plan to continue to study CSF neuropeptides in neuropsychiatric diseases. One approach which avoids the problem of spinal cord contributions to CSF levels of a peptide is to obtain ventricular CSF at autopsy. We plan to collaborate with Neal Cutler, MD, who will send us ventricular fluid from

Alzheimer's disease victims and suitable controls. We plan to measure CRF, SRIF, NPY and galanin. We plan to expand the study of peptide YY in bulimia by including plasma peptide YY measures in bulimics as well. Additional subjects may be studied in collaboration with David Jimerson, MD.

We intend to continue to study the increase in CSF NPY evoked by physostigmine. If we can make reliable measurements of the increase, we plan to study this phenomenon in Alzheimer's Disease.

We plan to expand the number of observations in the clonidine study. Additionally, we propose to study the ill relatives of patients who show the marker, to determine whether the blunted response segregates with illness in pedigrees. Lastly, we hope to use a cDNA probe for the alpha adrenergic receptor to evaluate linkage in some affective disorder pedigrees, using Southern blotting techniques.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02236-03 CNG

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Schizophrenia Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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Others:	E.S. Gershon	Chief	CNG, NIMH
	L.R. Goldin	Senior Staff Fellow	CNG, NIMH
	C.W. Dingman	Staff Psychiatrist	Chestnut Lodge
	I.D. Dauphinais	NRSA Fellow	CNG, NIMH
	M.E. Maxwell	Research Social Worker	CNG, NIMH
	J.R. Hamovit	Research Social Worker	CNG, NIMH

COOPERATING UNITS (if any)

Springfield Hospital; Chestnut Lodge; Clinical Center, NIH

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Section on Clinical Genetics

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.2

PROFESSIONAL:

1.4

OTHER:

0.8

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A study of clinical variables in multiplex families with schizophrenia was completed. In 53 of these families at least 2 siblings satisfied Research Diagnostic Criteria (RDC) for schizophrenia.

Brain imaging study: preliminary analysis of Magnetic Resonance Imaging (MRI) scans of these siblings revealed significantly reduced hippocampal area in schizophrenics as compared with related controls, suggesting that this may be a familial vulnerability factor.

Genetic marker studies: no association of Restriction Fragment Length Polymorphisms were found in genomic DNA from schizophrenics using several neuropeptide probes and probes for chromosome 11 (see Z01 MH 02237-03 CNG).

Protein polymorphisms in CSF were examined in 14 unrelated schizophrenics with a family history of schizophrenia. No abnormalities were found.

Project Description:

We maintain a structured evaluation system to screen referrals for chronic schizophrenia and to examine family pedigrees. We have recruited for multiplex families (families with more than one schizophrenic) throughout the United States with the help of the National Alliance for the Mentally Ill (NAMI).

Outpatient Clinic for the Treatment of Schizophrenia:

An outpatient clinic was established for pharmacologic treatment of schizophrenic patients who are members of multiplex families. Ten patients presently receive treatment as part of this clinic. Evaluations of other potential participants and their families continue.

Genetic Marker Studies:

Studies of potential protein markers in cerebrospinal fluid (CSF) have yielded negative results. Protein polymorphisms linkage analysis in lymphoblasts is in progress with Dr. Carl Merrill.

A cellular collection of affected-sib-pairs and pedigrees of schizophrenics has been established for DNA marker studies (see Z01 MH 00084-13 CNG).

Brain Imaging Studies:

Our latest brain imaging studies are with the use of the MRI. Twenty-four schizophrenic patients (11 sibling pairs and 2 unrelated siblings of schizophrenics) had significantly reduced bilateral medial limbic area compared with controls (N = 18).

	Schizophrenics (N = 24)	Controls (N = 18)	T	S
Total				
Anterior Limbic Complex				
(% Head Size)				
Left	1.47 \pm .31	1.67 \pm .33	1.96	.058
Right	1.41 \pm .33	1.63 \pm .36	2.01	.05
Posterior Limbic Complex				
Left	1.16 \pm .27	1.44 \pm .47	2.28	.03
Right	1.09 \pm .24	1.32 \pm .33	2.64	.01

Further analysis of these scans are in progress.

Significance to Biomedical Research and the Program of the Institute

Results from family, twin and adoption studies have strongly suggested a genetic component to the etiology of schizophrenia. In addition, environmental factors such as obstetrical complications, seasonality of birth, viral illness and a history of drug abuse have been implicated with the later development of schizophrenia. The exact nature of the inherited vulnerability and the interactions with environmental insults to the etiology of schizophrenia remains uncertain. Our registry of multiplex families is an important resource from which we can continue to study risk factors, and to evaluate the association of risk factors with biological markers that might be present in members of one family who have become ill with schizophrenia.

Our MRI study, now well underway, will be an important tool to further study the structural abnormalities demonstrated in postmortem studies of the brain in schizophrenia. In addition, the scanning of well siblings from the same families may establish these structural abnormalities as genetic vulnerability markers for the development of schizophrenia within these families.

The value of studying these families is further enhanced by our maintaining a collection of lymphoblast cultures for molecular genetic studies, linkage analysis and RFLP studies.

Proposed Course of the Project

We plan to perform MRI brain scans on well siblings in these families and to continue our evaluation of structural abnormalities in brain regions of interest (limbic regions, basal ganglia, temporal and frontal cortex) by computer analysis. We will continue to investigate the significance of risk factors in this population and our efforts to recruit new families into our program with emphasis on large pedigrees for molecular genetic studies.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02237-03 CNG

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	S. Detera-Wadleigh	Senior Staff Fellow	CNG, NIMH
Others:	E.S. Gershon	Chief	CNG, NIMH
	C. de Miguel	Visiting Fellow	CNG, NIMH
	B. SenGupta	Guest Researcher	CNG, NIMH
	W.H. Berrettini	Staff Psychiatrist	CNG, NIMH
	L.E. DeLisi	Staff Psychiatrist	CNG, NIMH
	L.R. Goldin	Senior Staff Fellow	CNG, NIMH

COOPERATING UNITS (if any)

Howard University

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Section on Clinical Genetics

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20892

TOTAL MAN-YEARS

4.2

PROFESSIONAL:

2.3

OTHER:

1.9

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Genetic linkage of affective disorders (AD) to c-Harvey-ras-1 (HRAS 1) and in insulin (INS) genes on chromosome 11 was examined using three pedigrees with bipolar illness. Our results indicate the absence of linkage from 0 to 15% recombination which contrasts with the finding of another group who found linkage to these markers in a large Old Order Amish pedigree. The reason for this discrepancy is not clear. Conceivably these contrasting findings favor etiological heterogeneity in AD.

Linkage to the color-blindness region, Xq28, was found to be absent in these same pedigrees using the St14 probe.

New restriction fragment polymorphisms (RFLP's) were detected at the gene regions encoding the gastrin releasing peptide (GRP), substance P, muscarinic receptor M-1, muscarinic receptor, M-4 and beta-adrenergic receptor. Analysis of the linkage relationships between AD and these genes are in progress.

New full length calmodulin cDNA for both human and rat have been isolated and sequenced. The nucleotide structures of these cDNAs are substantially different from those that have been reported. These results provide evidence for the presence of at least two actively transcribed calmodulin genes in these species.

A cDNA clone specific for the 87 kDa substrate of protein kinase C (PKCS-87) has been isolated from a rat brain expression library using a polyclonal antibody.

Other Investigators:

J. Gelernter	Medical Staff Fellow	CNG, NIMH
P. Gejman	Visiting Fellow	CNG, NIMH
T. Bonner	Senior Staff Fellow	LCB, NIMH
J. Patel	Visiting Associate	BPB, NIMH
D. Kligman	Staff Fellow	LCB, NIMH
S. Anderson	Medical Student	Univ. of Connecticut

Molecular Genetics

I. DNA Polymorphisms in Neuropsychiatric Diseases

Objectives

- a. To screen for restriction fragment length polymorphisms (RFLPs) at the loci of proteins with recognized neurobiological importance such as neuropeptides, receptors, enzymes involved in neurotransmitter synthesis and degradation and other proteins required for normal brain function.
- b. To use RFLPs in mapping the putative loci for affective illness and schizophrenia by linkage analysis.
- c. To search for association of polymorphic alleles of genes for DNA segments on the genome with schizophrenia and affective disorder.

Methods Employed

Genomic DNA is extracted and purified from lymphoblast cell lines or blood samples derived from patients, members of the patients' extended family and normal (control) individuals using standard procedures. The DNA is digested using a variety of restriction enzymes, the fragments fractionated on an agarose gel and eventually transferred to a nylon membrane by Southern blotting. Hybridization of the DNA with a radioactively labelled cDNA probe is done and the membrane is washed. The restriction pattern is revealed by autoradiography.

Major Findings

1a. The possibility of linkage of HRAS 1 and INS genes on the short arm of chromosome 11 to an AD locus was investigated in three North American pedigrees that have been collected by the Clinical Neurogenetics Branch. Haplotypes were derived from the HRAS 1 and INS alleles and because of the high polymorphism information content (PIC) value of these markers, all pedigrees were informative. Upon calculation of lod scores using a model of a dominant inheritance with high penetrance negative values lower than -2 were obtained at 0 to 15% recombination. These results indicate absence of linkage which is unlike the positive linkage obtained in a large Old

Order Amish pedigree by other investigators. This suggests that there is more than one gene involved in the etiology of AD. A cDNA probe for the tyrosine hydroxylase (TH) gene, which also maps to the HRAS 1 region of chromosome 11 was used to analyze the same AD pedigrees.

1b. Using the same 3 AD pedigrees and St14 (a generous gift of Dr. J.L. Mandel) as probe, linkage of the color-blindness region on xq28 was examined also. Linkage was negative in this region of the genome.

1c. The region of chromosome 7q22 to 7q35 was also studied for linkage to AD using the same 3 CNG pedigrees. Probes to the met oncogene and the gene for the Beta subunit of the T-cell receptor were employed. So far the results are indeterminate therefore more pedigrees need to be examined.

2. Two new RFLPs were identified at the substance P gene. The alleles found in all samples were analyzed to determine whether any of them is associated with either AD or schizophrenia. No association was detected, however, in the manic-depressive and schizophrenic patients studied although there is a tendency for homozygosity in both patient samples compared to normals. Similar results were found using the newly identified GRP RFLP. This tendency for homozygosity although not significant is intriguing.

3. Using genomic clones for the M1 and M4 muscarinic receptor genes which were isolated and characterized by Dr. T. Bonner, new RFLPs have been detected. Polymorphisms were also identified at the beta adrenergic receptor gene, a clone of which was kindly provided by Dr. Lefkowitz. Highly polymorphic minisatellite probes are currently being used to search for linkage in affective disorder and schizophrenia. These probes supplied by Dr. Jeffreys define approximately 40 loci in human genome, the location of which are not at present defined. Because they define so many loci simultaneously, these probes are valuable in the search for linkage.

II. Molecular Cloning of Human and Rat Calmodulin Genes

This project is being done in collaboration with Drs. Banani SenGupta and Felix Friedberg, Department of Biochemistry, Howard University.

Objectives

1. To determine the structure and organization of human calmodulin genes.

2. To determine the role of each of the active calmodulin genes in cellular regulation.

3. To examine the calmodulin locus for the presence of RFLPs and use these for association and linkage studies in schizophrenia and affective disorder.

4. To correlate the expression of calmodulin gene in various parts of the rat brain with development and aging.

Methods Employed

A rat brain lambda gtl1 cDNA library kindly provided by Drs. A. Dawsett, L. Fritz and N. Davidson, California Institute of Technology, was screened for positively hybridizing plaques using Xenopus laevis calmodulin cDNA probe from Dr. Igor Dawid, National Institute of Child Health and Human Development following standard procedures. Clone lambda rCB1 was isolated and purified. It was then digested with EcoRI and subcloned in pUC9 and M13 using standard methods. Sequencing was done following Sanger's dideoxynucleotide termination method.

A lambda gtl1 cDNA library constructed using mRNA from human teratocarcinoma cell line, NTera2D1, was kindly supplied by Drs. J. Skowronski, and M. Singer, National Cancer Institute. This human library was screened with the large EcoRI segment of lambda rCB1 in order to isolate calmodulin hybridizing plaques. One positive plaque was purified, subcloned in M13mp9 and sequenced by Sanger's method. Standard methods were followed in all the above procedures.

Major Findings:

A cDNA clone, lambda rCB1, encoding calmodulin was isolated from a rat expression library. The sequence was determined and compared to the structures of the previously described rat gene, lambda SC4 and lambda SC8. Faithful sequence conservation is observed in the coding regions of lambda rCB1 and lambda SC4, the bona fide gene. Both cDNAs encode identical amino acid sequence. Very limited sequence homology, however, is noted in the 3' untranslated segments of these clones. Surprisingly, when the lambda rCB1 nucleotide structure is compared to the processed ironless gene, lambda SC8, extensive sequence homology is found both in the coding and noncoding regions. The inferred protein sequences of lambda SC8 and lambda rCB1, however, are divergent. Using a fragment of lambda rCB1 to screen an expression library derived from a human embryonic cell line, calmodulin cDNA, lambda hCE1 was cloned and characterized. Comparison of the sequence of lambda hCE1 to calmodulin cDNA from human liver, hCWP, reveals substantial structural divergence. Strikingly poor homology is seen in the 5' and 3' noncoding regions but the coding portions were 85% homologous. Both lambda hCE1 and hCWP encode proteins of identical primary structure which is equivalent to the protein sequence deduced from lambda rCB1 and lambda SC4. Taken together these results suggest the existence of an additional actively transcribed calmodulin gene, not

previously identified, in each of the human and rat genomes. Transcripts of lambda rCB1 and lambda hCE1 were observed in all tissues examined indicating the absence of tissue specific expression. Calmodulin gene polymorphisms were detected using Taq I, Hind III and MspI.

III. Molecular Cloning of the Rat 87kDa Substrate of Protein Kinase C

This project is being done in collaboration with Drs. J. Patel and D. Kligman.

Objectives:

1. To determine the primary structure of PKCS-87.
2. To determine the gene organization of PKCS-87.
3. To study PKCS-87 cDNA in brain development and cellular transformation.
4. To isolate human PKCS-87 cDNA and genomic clones. To use these clones to search for RFLPs which will be employed in linkage mapping studies.
5. To determine the exact function of PKCS-87 in brain.

Methods Employed:

A rat brain lambda gt11-cDNA library, kindly provided by Drs. A. Dowsett, L. Fritz and N. Davidson, was screened with a PKCS-87 specific antiserum after induction of expression by IPTG. After several rounds of screening a single plaque was purified, amplified, subcloned and sequenced by the Sanger dideoxytermination method.

Subsequent screenings of the library were performed using oligonucleotides prepared on the basis of partial amino acid sequence of several tryptic peptides.

Major Findings

1. A cDNA clone containing a 600 bp insert was isolated using the antibody screening method from a rat brain expression library. Upon subcloning and sequencing the DNA segment was found to encode a peptide that is rich in alanine residues. This is an indication that the clone is authentic because PKCS-87 is composed of approximately 30% alanine. In order to obtain the full-length sequence other clones are being isolated using oligonucleotides as probes for screening.

Significance to Biochemical Research and the Program of the Institute:

Recent linkage studies on an Amish pedigree with bipolar illness have provided convincing evidence for the existence of a gene on chromosome 11 that is involved in the etiology of AD. This is a very important finding because the possibility of identifying a biochemical marker for AD is no longer remote. Our data as well as those of another group, however, suggest that in other pedigrees with AD other loci or genes different from the Amish disease gene might be involved. In view of this, one universal AD marker might not exist therefore the search for other loci should continue using more pedigrees and polymorphic probes. A similar approach to the study of the molecular genetics of schizophrenia must be undertaken since the underlying biochemical defect is not known in this disease. A by-product of these studies is the systematic exclusion of various regions on the human genome as potential genetic markers for affective disorder and schizophrenia since different kinds of cDNA probes will be used. Discovery of a DNA marker locus will have an immense impact on patient care as well as on the elucidation of the basic abnormality in these neuropsychiatric disorders.

Calmodulin is a calcium-binding protein with myriad functions in the cell. It is highly abundant in the brain. The molecule modifies the activity of various enzymes and receptors which are involved in neurotransmission, growth regulation and other basic functions of the cell. Other investigators have shown that there are at least two calmodulin-like genes in vertebrates. We recently found evidence that in rat and human at least two actively transcribed calmodulin genes exist which encode the same protein sequence. In order to study the regulation of these active genes we are isolating specific genomic clones. The genes will be studied to determine unique structural differences. We will attempt to ascertain whether one gene is involved in functions distinct from that of the other gene. This series of investigations is pertinent to the understanding of the role of calmodulin in development and neurological diseases.

Another second messenger system is linked to the calcium/diacylglycerol/phospholipid dependent protein kinase C (PKC). This enzyme is present in very high concentrations in the brain and plays a major role in the regulation of neuronal excitability. The main substrate of phosphorylation of PKC in the brain is PKCS-87 whose exact function is not known. The tumor promoting phorbol esters stimulate the phosphorylation of PKCS-87 at the same time influence the growth of neurons in culture, synthesis and release of neurotransmitters and the movement of ions across the membrane. PKC has been implicated also in the production of long term potentiation in the hippocampus, a phenomena which is hypothesized to be related to memory. Cloning of the cDNA and gene encoding PKCS-87 is important in the elucidation of the role this molecule plays in learning, memory and disease.

Proposed Course of Study:

In the following year we plan to screen other candidate genes for new RFLPs. These polymorphisms will be used to study association and linkage in schizophrenia and affective disorder. Genomic blots derived from additional AD and schizophrenia pedigrees and sib-pairs collected by the CNG Branch will be examined for linkage to the INS-HRAS1-TH region. Other probes with high PIC value will be used also. Preparation of genomic DNA from the new collection of AD and schizophrenia pedigrees and schizophrenia sib-pairs will be continued.

The structures of the actively transcribed calmodulin genes in humans will be determined. Regulation of each gene will be studied as it relates to development and the cell cycle. RFLPs will be determined at the calmodulin loci so that it can be used as probe in linkage studies.

The full-length cDNA for the PKC substrate protein, PKCS-87, will be derived from individual clones isolated using both antibody and oligo-nucleotide screening methods. The expression of PKCS-87 will be examined in the presence of cellular stimuli. Human PKCS-87 will be isolated also for RFLP linkage studies.

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Detera Wadleigh, S.D., Anderson, S., and Spindel, E.R.: A frequent PvuII RFLP of the human gastrin releasing peptide gene. Nuc. Acids Res. 15: 375, 1987.

SenGupta, B., Friedberg, F. and Detera-Wadleigh, S.D.: Evidence for the presence of multiple human calmodulin genes. Fed. Proc. 46: 2001, 1987.

SenGupta, B., Friedberg, F., and Detera-Wadleigh, S.D. Molecular analysis of human and rat calmodulin cDNA clones: Evidence for additional active genes in these species. J. Biol. Chem., in press.

Detera-Wadleigh, S.D., de Miguel, C., Berrettini, W.H., DeLisi, L.E., Goldin, L.R. and Gershon, E.S.: Neuropeptide gene polymorphisms in affective disorder and schizophrenia. J. Psych. Res., in press. Also to appear in Gershon, E.S., Cloninger, C.R., Propping, P., DeLisi, L.E., Hippus, H. and Bondy, B. (Eds.): Genetic Research in Psychiatry. Oxford, Pergamon Press, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00935-20 CNG

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Plasmids and Small Genomes in Human Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

PI: C.R.Merril Chief, Biochemical Genetics Section CNG, NIMH
 Others: L. Mitchell Staff Fellow CNG, NIMH
 D. Rath Staff Biologist CNG, NIMH
 B. Budowle Chief, Forensic Sci. Research FBI, Academy

COOPERATING UNITS (if any)

Forensic Science Research Group, FBI Academy, Quantico, Virginia

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Biochemical Genetics Section

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.25

PROFESSIONAL:

1.25

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Numerous studies of the human mitochondrial genome have proven the utility of this small, naturally occurring plasmid in elucidating the relationship between populations. The unique perspective provided by investigations of this genome depend on the maternal inheritance pattern of this plasmid and its relatively high rate of mutation. These characteristics of the mit-genome have suggested that it may be useful in identifying individuals and in providing information on the geographic origin of the individuals maternal ancestors. A collaborative study is underway with the FBI Forensic Science Research Group to determine the feasibility of employing the mitochondrial genome in forensic applications. The methods that are being developed in this collaborative study are also being used to examine the rate of mutation of the mit-genome in post-mitotic tissues, such as the central nervous system. Additionally, these methodologies are being used to study a group of diseases that display non-Mendelian maternal inheritance patterns, which may be due to mutational events in the mitochondrial genome.

PROJECT DESCRIPTION

Mitochondrial DNA was initially discovered more than 20 years ago. Since then, it has been determined that this DNA molecule represents a unique genome 16.5 kb in length which exists in a closed circular structure. Additional research has also shown that mitochondrial DNA (mit-DNA) is monoclonal in origin and has a high mutation rate (5 to 10 times that of comparable nuclear genes). This mutation rate may be due to the apparent lack of both replicative and post-replicative DNA repair mechanisms in the mitochondria.

The lack of repair mechanisms suggested that the human mitochondrial genome might serve as a good indicator concerning the accumulation of mutational events in postmitotic somatic tissues and in the germ line. Studies of somatic mutational events may provide insights into pathophysiological processes while germ line mitochondrial mutational events have already proved useful in anthropological studies and they may further our ability in establishing forensic individuality.

The section has been developing methods to examine the intra-individual somatic heterogeneity of the mitochondrial genome in the central nervous system. The brain, a highly aerobic tissue, relies heavily on its mitochondrial population for critical metabolic processes. Many of these metabolic processes create free radicals, which have the potential to cause mutational events by their interactions with DNA. The close proximity of the mitochondrial DNA to the sites of production of free radicals, coupled with the apparent lack of DNA repair mechanisms, in the organelle suggests that somatic mutations should accumulate in postmitotic tissues with aging and in certain disease processes.

Studies of intra-individual somatic cellular mitochondrial heterogeneity have been initiated by purifying mit-DNA from human brain tissue samples obtained at autopsy. The mit-DNA has been digested with restriction endonucleases and attempts have been made to clone a 636bp fragment from one of the most mutational labile regions of the mitochondrial genome, the hypervariable region of the D-loop. Despite numerous attempts only one clone has been obtained which contains the fragment of interest, despite several repetitions of the protocol. To detect a reasonable mutation rate by the RNA-DNA duplex procedure, it is necessary to screen 200+ independent clones for base pair mutations. Since this single clone is inadequate for our study and due to the apparent refractory nature of this area of the human mitochondrial genome to cloning [Anderson et al. noted difficulties in cloning this region during the first sequencing exercise of the human mit-genome], a second area, the cytochrome oxidase

subunit II, has been selected for investigation. The D-loop clone has been stored for possible use in future studies.

The region coding for the cytochrome oxidase subunit II has an intermediate degree of sequence variability compared to the hypervariable region of the D-loop. The fragment selected from the cytochrome oxidase region is 548bp in length and encompasses 80% of the entire gene. At present, this 548bp segment has been cloned into a pUC vector and then subcloned into a pSP vector. The pSP vector contains a SP6 promotor which allows for the synthesis of RNA probes homologous to the cloned insert. These probes will then be hybridized to DNA from 200+ individual clones obtained from the same tissue as the original clone. If any base substitutions are present, they will be detected by cleavage with ribonuclease A at the mismatched regions in the RNA:DNA duplexes followed by denaturing gel electrophoresis. This method will detect greater than 70% of the mismatches present and can be used more efficiently to examine the mitochondrial genome in an individual for the accumulation of mutational events than conventional sequencing technologies. In this manner, the variations which may have accumulated through physiological or pathophysiological events over the course of a lifetime as a result of random mutations in a specific region of an individual's brain can be evaluated.

The section is also developing procedures to permit the direct sequencing of plasmid DNAs, such as the human mit-DNA. These sequencing techniques will permit a detailed examination of any mutational events discovered in the screening program described above. Direct sequencing with elimination of the need for cloning should save more than half the time currently needed to determine a DNA sequence. Development of such methods may aid in determining the degree nucleotide sequence heterogeneity within and between individuals.

The section has concentrated its attention to three regions; the D-loop which is considered to be the most variable region in the entire human genome, an adjacent t-RNA region which should be highly conserved, and the cytochrome oxidase subunit II, which has an intermediate degree of variability. Clones of these regions from a number of individuals are being prepared for sequence analysis to verify the levels of inter-individual variability previously reported in the literature. These clones are also serving as the initial source of DNA for the direct sequencing studies since they provide control sites for the initiation of Sanger dideoxy sequencing.

With the assistance of Dr. M. Brownstein, we have acquired a number of synthetic DNA oligomers complementary to the three previously mentioned mit-DNA regions; 5'-GATTCCGCTCATCTATT-3' for the D-loop,

5'-TTGACTGTAATGTGCTATGT-3' for the D-loop and proline tRNA, and 5'-ACAGCTCATGAGTGCAAGAC-3' for the cytochrome oxidase II regions are representative examples. These oligomers are being used as primers for the direct sequencing strategy. Partial mit-DNA sequences have been produced with this technique. A source of difficulty in achieving an operational method with this approach has been attributed to the ribonucleotide residues which are contained within the mit-genome. Mitochondrial DNA contains an average of five ribonucleotides per molecule and denaturation of these molecules with alkali prior to the sequencing reaction often breaks the molecules at the random locations of these ribonucleotides, producing anomalies in the sequencing process. A test concerning the effects of exposure to alkali on mit-DNA has shown that exposure for periods as short as 30 seconds can result in the obliteration of an electrophoretic mitochondrial band which is clearly present in the untreated sample. Alternative means of producing single stranded denatured DNA are being investigated to optimize the conditions for the sequencing reactions.

Significance to Biomedical Research and the Program of the Institute:

The mitochondria's relatively high mutation rate and apparent lack of DNA repair mechanisms may result in the accumulation of errors in the mitochondrial DNA which, in turn, may be associated with degenerative alterations in highly aerobic tissues. Additionally, the methods under development may be useful in studying some of the maternally inherited diseases, such as Leber's optic atrophy. Direct sequence analysis may permit the identification of precise nucleic acid mutations in the mitochondrial genome which may be responsible for certain of these diseases.

Proposed Course of the Project:

The direct DNA sequencing and refined hybridization methodologies which are being adapted to study the mitochondrial genome should enable us to identify and rapidly sequence portions of mitochondrial genomes which demonstrate interesting variations. By examining large numbers of independent clones from specific regions of the mitochondrial genome it should be possible to determine whether or not mutational events accumulate in this genome with aging, or during disease processes, and whether such changes are tissue specific. Furthermore, these techniques should permit enhanced examinations of inter-individual genomic variation. Such inter-individual genomic variations may prove useful for the establishment of individuality and they may also provide some clues as to the maternal geographic origin of forensic samples. The section has

established a collaboration with the Federal Bureau of Investigation's Forensic Science Research Group to test the feasibility of using mitochondrial and nuclear DNA's isolated from forensic samples for identification purposes.

The direct mit-DNA sequencing technique will also be utilized in studies of a group of diseases which are inherited in a maternal non-mendelian pattern. Some of these diseases may be due to mutational events in the mitochondrial genome. We have initially chosen to study Leber's optic atrophy, a hereditary blindness which is maternally inherited. Fifty percent of affected females pass the disease to their children and an additional 40% of the offspring are carriers. To date there has not been a report of paternal transmission. In collaboration with Dr. Kaiser of the NEI, we have obtained platelets from a family with this disorder, including both affected and normal individuals. The platelets were collected by the NIH Blood Bank and we have isolated the mit-DNA from them for future analysis.

Publications None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00941-07 CNG

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Genetics and metabolic diseases.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C.R.Merril Chief, Biochemical Genetics Sect. CNG, NIMH
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COOPERATING UNITS (if any)

NIMH, NINCDS, USUHS, Vanderbilt University, Harvard Medical School, California Institute of Technology, Baylor College of Medicine, University of Goteborg Sweden, US NAMRU-11 (Philippines).

LAB/BRANCH

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TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

2.5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Disease-associated cerebrospinal fluid (CSF) protein changes that were previously identified by this group have been further investigated: The presence of two 30,000 MW proteins in CSF of Creutzfeldt-Jakob disease (CJD) patients have been studied prospectively for their usefulness in the diagnosis of 70 nationally and internationally referred cases: no false positives or false negatives were found in the 30 cases that have so far come to autopsy (this has included 3 cases of in-vivo diagnosis of growth hormone transmitted CJD). Further characterization of abnormal CSF proteins have led to purification of 8 proteins for structural analysis, and one of these, a glycoprotein, has been partially sequenced. Four peptide fragments, obtained from a tryptic acid digest, 8 to 18 amino acids in length have been sequenced from this protein. These sequences represent a new protein that has not previously been characterized in the protein sequence data banks. This glycoprotein has been shown to be quantitatively altered in schizophrenia, Perkinson's Disease and multiple sclerosis.

Two dimensional electrophoretic survey studies of brain have been initiated with inbred strains of mice, with a view to developing a protein reference map for both genetic murine disorders and human brain disorders. Similar studies have led to the observation of protein alterations in learning mutants of Drosophila, and T lymphocytes infected with the human immunodeficiency virus.

Continued efforts to investigate protein detection methods has permitted us to develop a physical explanation for the production of specific colors by protein stained with certain silver stains. Electron microscopic studies coupled with computerized image analysis provided evidence that the colors are formed by light scattering which is caused by the presence of silver grains of specific sizes in the gel. Silver grains in yellow bands had an average size of 29.6nm while those in blue bands were 71.6nm in diameter.

Collaborators:

L.DeLisi	Assoc. Professor	SUNY, Stoneybrook
D.Dauphonais	Staff Fellow	CNG NIMH
E.Gershon	Chief, Clinical Neurogenetics Branch	NIMH
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D.Asher	Senior Staff	CNSS NINCDS
D.C.Gajdusek	Chief, Lab. of Central Nerv. Sys. Studies	CNSS NINCDS
P.Brown	Neurologist	NINCDS
E.F.Torrey	Staff Psychiatrist	St. Elizabeths Hosp. Wash., D.C.
R.S.Burns	Assoc. Professor	Vanderbilt Univ.
A.Percy	Assoc. Professor	Baylor College of Med.
B.Hagberg	Chairman, Dept. of Ped.	Univ. of Goteborg, Sweden
G.Watt	Med. Director	Navy/Army Med. Res. Unit 2, Philippines
T.Folks	Senior Staff Fellow	LIG NIAID
I.Hay	Professor of Virology	USUHS
R.Brady	Chief, DMN	NINCDS
L.Hood	Chairman, Biology Dept.	California Inst. of Tech.
S.Kent	Scientist	California Inst. of Tech.
R.Aebersold	Scientist	California Inst. of Tech.
R.Sidman	Prof. Neuropath	Dept. Path. Harvard Med. School
P.Neumann	Staff Fellow	Dept. Path. Harvard Med. School
A.Steven	Senior Staff	LPB NIAMS

Objectives:

Investigations of diseases which affect the central nervous system, such as schizophrenia, would greatly benefit from a knowledge of the genes and proteins that are active in the human brain. The technologies that will permit the establishment of the complex databases required for this task are now becoming available. The development of high resolution protein separation technologies [eg. two-dimensional electrophoresis], highly sensitive protein detection methods [such as silver staining] and microsequencing methods [eg. as those currently under development in Leroy Hood's Cal-Tech laboratories] will provide the opportunity of separating, visualizing, and identifying most of the protein gene products present in human tissues, and body fluids. These technologies will also permit studies involving global relationships between these protein gene products. Furthermore, the knowledge of even partial sequences of each of the proteins will permit the interlinking of this protein database with the genomic DNA databases which are currently being developed.

Within the next decade the complete human genome will be mapped in some detail, and the efforts to completely sequence the genome may also be well advanced. The establishment of protein data-banks will complement the genomic mapping and sequencing endeavors.

The section has initiated a brain and spinal fluid protein database program. Current technology permits the visualization of 1,000 proteins in 40ul of unconcentrated spinal fluid [CSF]. Of these 1,000 proteins 80 have been identified by co-electrophoresis and western blotting [immunological methods]. Partial amino acid sequences have been determined, from proteins isolated from the gels, for two of the CSF proteins. Of particular interest are 5 CSF proteins which have only been observed in disease states. The immediate strategy is to obtain partial sequences for these disease specific proteins, and to construct synthetic peptides to raise antibodies for immunological assays, and synthetic oligonucleotides to screen DNA libraries for the origin of these proteins.

The construction of a mammalian brain protein databank has been advanced by the section's studies of protein changes in the nervous system of mutant mouse stocks. These studies may provide identifying homologs of human diseases, and they may also help us achieve a greater understanding of the molecular processes of the nervous system. From a baseline of normal proteins of regions of brain, spinal cord and peripheral nerve, identification of mutant-specific protein changes can be made by a combination of protein biochemistry and classical mouse genetics/breeding systems.

The section also continues to invest in the development of methods to separate, visualize and identify proteins. The introduction of silver-staining by this section, to detect electrophoretically separated proteins in polyacrylamide gels has provided a method that, with the most responsive proteins, is more sensitive by a factor of ~100 than Coomassie Blue, the most commonly used organic stain. With silver staining, most proteins take on a brownish hue. However, under appropriate conditions, certain proteins have been found to exhibit distinct and vivid colors. Yellow, blue, red and green bands have all been observed. Colorability is a property with considerable analytical potential, in that it may become possible to infer chemical properties of proteins on the basis of their propensities for coloration upon silver-staining. Such information would considerably enhance the analytical capabilities of gel electrophoresis, which for the most part have been restricted to estimates of molecular weights and isoelectric points. To help realize this potential, we have investigated the physical basis of the colorability of proteins.

Subjects:

All the serum, tissue and spinal fluid samples were obtained from patients who were diagnosed by our clinical collaborators with nationally recognized diagnostic criteria. Inbred mouse and Drosophila strains were obtained from our collaborators at the Harvard Medical School.

Laboratory procedures:

The laboratory employs numerous protein purification procedures, including chromatography, and electrophoresis methodologies. The electrophoretic methods include both one and two dimensional electrophoresis. Proteins are detected by silver, dye and immunological staining.

Progress in the computer-assisted analysis and image processing of 2D gels has been enhanced by the establishment of a collaborative effort with Mark Miller and his co-workers in the NCI. The Image Analytics Corporation (IAC) [Delaware] computerized laser gel scanner has proven to be fairly reliable and it has provided linear quantitative data for gel analysis. The collaborative arrangement with the NCI is providing higher level gel matching programs than IAC scanner could achieve without their support.

Electron microscopic analysis of colored silver stained gels was performed in collaboration with Alasdair Stevens and his colleagues in the NIAMSD. Silver stained bands were dissected from gels and examined with a Philips EM300 transmission electron microscope at a magnification of 16,000X.

MAJOR FINDINGS:

1. CSF protein studies:

(a). Studies in recent years with our colleagues in the NINCDS have resulted in the identification of two abnormal 30,000 MW CSF proteins in patients with Creutzfeldt-Jakob disease. This year, we initiated a prospective differential diagnosis of study of patients with suspected CJD dementia. CSF from 70 national and international cases of suspected CJD have been referred to us at various stages of their illness, and at present, autopsies have been obtained in 30 of these patients. The two abnormal proteins have been identified in all patients in this study that have been found to have pathology consistent with CJD. No false positives or negatives have occurred so far. Three atypical cases of CJD originating in the recipients of cadaver-derived human pituitary growth hormone were diagnosed in-vivo, two of whom have been pathologically verified.

The two abnormal CJD diagnostic proteins (numbered 130 and 131) have been purified by two dimensional electrophoresis. The purified proteins have been analyzed, following tryptic digestion, for their peptide patterns. Separation of the tryptic digested products by HPLC produced similar chromatographic profiles for each of the two abnormal proteins, suggesting that proteins 130 and 131 are very similar. They may only differ by one or a few amino acid substitutions or by a post-translational modification. Additional quantities of these proteins are being purified to extend these studies to obtain partial amino acid sequences.

(b). In conjunction with our collaborators in NIMH, NINCDS, NIAID, USUHS, NAMRU 11, Baylor College of Medicine, Goteborg/Sweden, and Vanderbilt University, we continue to characterize CSF proteins that have been identified as being altered in diseases of the nervous system. The work is at the stage where many of these proteins are in varying stages of purification. One protein (25,000 MW) is present in varying degrees of glycosylation in the normal CSF, and is quantitatively altered from normal in several diseases studied: Parkinson's disease, schizophrenia and multiple sclerosis. After purification and tryptic digestion, the amino acid sequence of four peptide fragments from this protein have been obtained in collaboration with our collaborators in Leroy Hood's laboratory at Cal Tech. The sequences range from 8 to 18 amino acids in length. They form part of the 25,000 MW protein and are different from all known sequences in the protein sequence data banks. Studies are in progress to synthesize peptides and oligonucleotide probes to further characterize this major CSF protein (circa 5% of the total CSF protein).

2. Studies on genetic models of disease:

(a). In collaboration with Dr. Brady (NINCDS), we have identified protein changes in the fly heads of a number of learning mutants of Drosophila melanogaster. These proteins are being further characterized.

(b). In collaboration with Dr. Folks (NIAID), we have characterized, in preliminary studies, many protein alterations in T lymphocytes infected with the human immunodeficiency virus compared to uninfected cells. We are pursuing this to identify perturbations in the cell metabolism that are caused by the virus as compared to both normal cells and other retroviruses.

(c). In collaboration with Sidman and Neumann (Harvard Medical School), we have initiated experiments on protein patterns in the nervous system of inbred strains of mice. The strategy is to create a baseline of normal proteins in different brain regions under different preparative conditions: a protein brain map. This will allow a future comparison with protein patterns in mutant mice, and will afford an animal model to study technical questions that may be relevant to human genetic diseases affecting the CNS.

3. Detection of Proteins

A priori, two mechanisms appeared most likely to be responsible for color specificity of silver stains observed with certain proteins: (i) the development of microscopic silver grains whose size dictates the color; and (ii) the bonding of silver atoms to functional groups on the proteins to form specific complexes. In the former case, color would be produced by diffractive scattering of light, and in the latter case, by conjugate bond systems analogous to those in naturally-occurring colored proteins.

To distinguish between these hypotheses, we have compared the size distributions of silver particles visualized in thin section prepared from protein gel bands of different colors. Mechanism (i) predicts a pronounced correlation between visible color and the diameters of the silver grains, whereas, according to mechanism (ii), the silver-containing entities responsible for color generation would always be on the sub-molecular scale (i.e. $<1\text{nm}$). Samplings of the silver grains typical of a yellow-staining protein (human serum albumin) and a blue-staining protein (a minor satellite band associated with apolipoprotein A) demonstrated grains that were bigger than the 5-15nm micro-grains found sparsely distributed throughout the (brownish) background areas of the gel. In the yellow band, these grains are markedly smaller in diameter (20-40nm, $u=29.6\text{nm}$) and more homogeneous than those in the blue band (40-100nm, $u=71.6\text{nm}$).

Based on these and other observations, we envisage the following mechanism for colored silver-staining of proteins: the presence of certain functional sites or groups will, under appropriate development conditions, nucleate globular silver grains that grow to sizes whereby their light-scattering properties emphasize specific colors. Within this overall scenario, the precise nature of the nucleation event(s) as well as of other aspects of the process have still to be determined, although it has been established that a high incidence of certain amino-acids in a protein - notably basic residues or cysteines - will strongly influence its colorability.

It is intriguing that, in contrast to the familiar sheen of bulk metallic silver, colloidal suspensions of microscopic silver particles may produce many different colors. In this respect, colored silver-staining of proteins appears to be broadly analogous to silver-based color photography.

Significance to Biomedical Research and to the Program of the Institute:

The development of protein databases based on their electrophoretic separation with two-dimensional electrophoresis [which potentially provides information on their mass, charge and quantitation] could facilitate research on the pathophysiology of diseases of interest to the NIMH, such as schizophrenia.

The section has initiated efforts, including a number of pilot experiments, to develop a brain database. We have, in collaboration with David Jacobowitz, examined protein patterns in 25 different regions of the rodent brain. In these studies, we were able to demonstrate brain region-specific protein patterns. We have also found disease specific proteins in human spinal fluid and are currently collaborating with Dr. Hood's Cal Tech. group to sequence these proteins so that immunological and DNA probes can be constructed. These probes will serve as useful diagnostic aids and will help to further our understanding of these diseases.

The section's continued efforts to improve protein separation and detection methods has led to an enhanced understanding of why certain proteins produce specific colors with silver staining. Color is a property with considerable analytical potential. As we learn more about the chemistry of silver staining it may become possible to determine chemical properties of proteins based on the proteins ability to produce a specific color or staining reaction with silver. This type of information would considerably enhance the capabilities of gel electrophoresis, both as an analytical and diagnostic tool.

Proposed course of the Project:

The section plans further development of brain and spinal fluid protein databases based on electrophoretic separation technologies and protein detection by silver staining. The section's computerized microdensitometry equipment, has proven insufficient for the analysis of the large amount of data that is currently being collected for these databanks. It is hoped that the current up-grading of the section's computer facilities coupled with the collaborative arrangement that has been established with image processing groups in the NCI will alleviate these problems. Brain proteins that display quantitative or qualitative alterations after viral infection or after the administration of certain drugs, such as the neuroleptic (antischizophrenic) drugs, chlorpromazine and haloperidol, may warrant detailed investigations. Knowledge of region-specific proteins could also permit molecular investigations that would complement the current PET scan studies of abnormal brain metabolism.

Microsequencing of disease specific proteins and physiologically important proteins will be conducted in collaboration with Leroy Hood's Cal Tech. laboratory. The sequences obtained will be used to determine whether the disease specific proteins are endogenous or exogenous [possible viral origin]. They will also provide information for the construction of peptides to stimulate antibody production [for diagnostic assays] and DNA oligonucleotide probes [to determine their origin]. Furthermore, the sequences of the proteins of interest will permit cross-referencing with the genomic DNA databases.

The section plans to continue to develop methods to enhance our ability to resolve and identify individual proteins from tissues and body fluids. Currently, it is often possible to separate and visualize up to 5,000 proteins on a single electrophoretic gel from a tissue sample with high resolution 2DE and silver staining.

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Patent Pending:

NIH # E-191-87 diagnostic test for Creutzfeld-Jakob Disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01836-09 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

GABA/Receptors in the Central Nervous System: Biochemistry to Behavior

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: S.M. Paul, Chief, NS, NIMH

Others: J. Crawley	Sr Staff Fellow	NS, NIMH
S. Cottingham	PRAT Fellow/Guest Researcher	NS, NIMH
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P. Suzdak	PRAT Fellow	NIGMS, NIMH
A. Morrow	PRAT Fellow	NIGMS, NIMH
S. Deutsch	PRAT Fellow	NIGMS, NIMH

COOPERATING UNITS (if any)

Laboratory of Bioorganic Chemistry, NIADDK; Section on Molecular Pharmacology, NS, NIMH; Unit on Molecular Neurogenetics, NS, NIMH; Pharmacology Research Associate Training Program, NIGMS; LSU School of Medicine, New Orleans, LA

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Preclinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

3.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Benzodiazepines interact with a specific neuronal membrane receptor to initiate a series of neuronal events resulting in an enhancement of GABA-mediated chloride permeability. The latter results behaviorally in the major pharmacological actions of benzodiazepines, namely their anxiolytic, anticonvulsant, hypnotic and muscle relaxant actions. In addition to benzodiazepines, a variety of sedative/hypnotic agents of the minor tranquilizer class (e.g. the barbiturates) appear to interact with one or more components of the benzodiazepine/GABA receptor complex, and thus the latter has been proposed as a common site of minor tranquilizer action. Several aspects of the benzodiazepine/GABA receptor complex are currently being studied. Recent work has employed an in vitro system for measuring GABA receptor-effector coupling in a subcellular preparation from rat brain (the synaptoneurosomes). This technique has greatly facilitated studies on the regulation of the GABA receptor-coupled chloride ion channel. Using this method, we have studied the interaction of ethanol with the GABA receptor complex and have found that ethanol, related short-chain alcohols and several anesthetic agents are capable of stimulating this receptor and at pharmacologically-relevant concentrations. In related studies we have identified a novel imidazobenzodiazepine, Ro15-4513, which blocks both the in vitro effects of ethanol on GABA receptor-mediated ³⁶Cl⁻ uptake as well as many of the behavioral effects of ethanol. Chronic administration of ethanol to rats results in a decrease in GABA receptor-mediated ³⁶Cl⁻ uptake in synaptoneurosomes, an effect that is reversible since it is not observed after the ethanol withdrawal syndrome. In other studies we have examined the use of the radiolabelled benzodiazepine receptor antagonist Ro15-1788 for measuring benzodiazepine receptors in vivo. Our results have validated the suitability of this technique and have demonstrated significant effects of barbiturates, naturally-occurring steroid hormones, ethanol and "stress" on benzodiazepine receptors in vivo.

PROFESSIONAL PERSONNEL:

A. Weizman	Guest Researcher	NS, NIMH
R. Weizman	Visiting Scientist	NS, NIMH
F. Vocci	Guest Researcher	NS, NIMH
B. Martin	Visiting Scientist	NS, NIMH
E. Ginns	Neurologist/Biochemist	NS, NIMH
P. Skolnick	Pharmacologist	LBC, NIADDDK
L. Miller		LSU

PROJECT DESCRIPTIONObjectives:

1. To characterize the interaction of anxiogenic and anxiolytic compounds with the benzodiazepine/GABA receptor complex at the molecular/cellular, neurophysiologic and behavioral levels.
2. To understand the mechanism(s) responsible for the stress-induced "sensitization" and drug-induced desensitization of this receptor complex (including the possible involvement of steroid hormones); and to unravel whether changes in the ionophore underlie the development of tolerance to sedative/hypnotic/anxiolytic drugs.
3. To use the GABA receptor-coupled Cl^- ion channel complex as a model of other neurotransmitter-gated ion channels.
4. To explore basic neurochemical mechanisms of anxiety, fear and stress as they relate to the many clinical and medical problems associated with stress.

Methods Employed:

(See: 1986 Annual Report, Project Number Z01 MH 01836-08 NS, GABA/Receptors in the Central Nervous System: Biochemistry of Behavior)

Major Findings:

Agents that perturb one or more of the components of the benzodiazepine-GABA receptor chloride ionophore complex (e.g., benzodiazepines, GABA and GABA mimetics like muscimol and barbiturates) have been shown to increase chloride conductance in both electrophysiological preparations and intact cells. Attempts to develop a quantitative measurement of chloride flux in a "cell-free" preparation (in order to explore the functional relationships between the binding sites associated with the receptor complex) have generally been unsuccessful. We have developed a method for measuring $^{36}\text{Cl}^-$ flux in "filtered synaptoneurosomes." The filtered synaptoneurosomes preparation was employed because it has been shown to contain both presynaptic nerve endings and attached postsynaptic densities. We have previously shown that barbiturates, including pentobarbital, cause a concentration dependent increase in the efflux and uptake of $^{36}\text{Cl}^-$ efflux from synaptoneurosomes which is reversed by the chloride ionophore antagonist,

picROTOXIN. A good correlation ($r = 0.90$, $p < 0.01$) was observed between the potencies of a series of barbiturates in increasing $^{36}\text{Cl}^-$ efflux from preloaded synaptoneurosomes and their anesthetic potencies in mice. Barbiturates also potentiate the stimulation of $^{36}\text{Cl}^-$ uptake induced by GABA agonists such as muscimol. In addition to barbiturates, a number of naturally-occurring substances including the A ring reduced metabolites of progesterone and deoxycorticosterone (3 alpha 5 alpha dihydroprogesterone and tetrahydrodeoxycorticosterone) have been found to be potent barbiturate-like modulators of the GABA receptor-gated chloride ion channel. The latter have also been shown to have anxiolytic and hypnotic effects in rats and mice.

Work over the past several years has documented that both benzodiazepines and barbiturates stimulate $^{36}\text{Cl}^-$ efflux or uptake via a pharmacologically-relevant GABA_A receptor. Since the latter drugs show cross-tolerance and cross-dependence with alcohol we have further examined the effects of ethanol (the most commonly used anxiolytic/hypnotic/intoxicant) on the GABA receptor coupled Cl^- ion channel. Our data demonstrate that ethanol at concentrations from 20 to 100 mM stimulate $^{36}\text{Cl}^-$ uptake via the GABA_A receptor, since this effect is blocked by the GABA_A receptor antagonists bicuculline and picROTOXIN and not by a variety of other neurotransmitter receptor antagonists. In addition to ethanol, many short-chain alcohol and anesthetic agents tested stimulate $^{36}\text{Cl}^-$ uptake. These data suggest that ethanol (and related alcohols) produce at least some of their behavioral effects via an interaction with the GABA receptor-coupled Cl^- ion channel and most likely by altering the membrane (lipid/protein) microenvironment of the receptor, rather than directly binding to the receptor protein itself. In related experiments we have found that the imidazobenzodiazepine Ro15-4513, which is a derivative of the benzodiazepine antagonist Ro15-1788, blocks the ability of ethanol to stimulate $^{36}\text{Cl}^-$ uptake *in vitro*. Moreover, Ro15-4513 fails to block either muscimol- or pentobarbital-stimulated $^{36}\text{Cl}^-$ uptake at concentrations ≤ 1 μM . In behavioral studies, Ro15-4513 also blocks the effects of low (1 g/kg) as well as moderate (2 g/kg) doses of ethanol in rodents; but not higher (≥ 4 g/kg) doses of ethanol. The effects of Ro15-4513 in blocking both ethanol-stimulated $^{36}\text{Cl}^-$ uptake *in vitro* and ethanol-induced intoxication *in vivo* were not mimicked by the full inverse agonist BCCE or the partial inverse agonist FG-7142. The latter finding suggests that Ro15-4513 has "selective" anti-alcohol actions heretofore not observed with other benzodiazepine receptor inverse agonists. Together, these data support the hypothesis that many of the neuropharmacological effects of low to moderate doses of alcohol are mediated via augmentation of central GABAergic neurotransmission. Chronic administration or treatment with sedative/hypnotic drugs (barbiturates, benzodiazepines, ethanol) is generally associated with the development of pharmacodynamic tolerance. However, the mechanisms for this tolerance are obscure. Recently, we have shown that chronic barbiturate or ethanol administration to rats results in a 25-35% decrease in the apparent V_{max} of muscimol-stimulated $^{36}\text{Cl}^-$ uptake in cerebral cortical synaptoneurosomes. Following chronic ethanol administration, this "subsensitivity" in GABA receptor function is highly correlated to the blood ethanol level and with the development of the withdrawal syndrome.

Despite strong evidence that suggests benzodiazepine receptors mediate the antianxiety (anxiolytic) actions of the benzodiazepine (e.g., diazepam,

chlordiazepoxide) and related compounds, the physiological function(s) of these sites is unclear. Several lines of evidence now suggest that these receptors may play a role in the control of arousal and the central "stress" response. We have recently demonstrated that several stressors (forced ambient temperature swim, brief immersion in ice water, and food deprivation for the first three hours of the dark cycle) elicit a rapid and robust change in [^3H]benzodiazepine binding that is observed only in the presence of Eccles' permeable anions (e.g., chloride, iodide and bromide ions). These differences are manifest as an increase in the apparent affinity of [^3H]flunitrazepam, with no significant differences in the maximum number of binding sites (B_{max}) between the groups. Both an increase in the maximum enhancement of [^3H]flunitrazepam binding in response to optimum concentrations of halide ions (E_{max}) and an increased sensitivity to halide ions (reduced EC_{50}) were observed in response to stress. These results suggest that acute exposure to stress effects either the coupling of the chloride ionophore and benzodiazepine receptor, or that the chloride ionophore itself in some way modified. Further, the ability of muscimol to stimulate $^{36}\text{Cl}^-$ uptake *in vitro* is enhanced in synaptosomes prepared from "stressed" rats when compared to stress-habituated controls. The effects of swim stress in potentiating muscimol-stimulated $^{36}\text{Cl}^-$ uptake were attenuated in adrenalectomized animals suggesting a "permissive" role for glucocorticoids in mediating this stress response. Recently, using an *in vivo* technique for labelling the benzodiazepine receptor (using [^3H]Ro15-1788) we have observed biphasic effects of swim stress on benzodiazepine receptor occupancy. Acute swim stress increases specific [^3H]Ro15-1788 binding immediately (≤ 1 hr) after stress whereas specific binding is decreased in many brain regions 24 hours after stress. Similar acute effects were observed after "defeat stress" another form of social stress. The possible role of adrenal steroid secretion in mediating the "delayed" effects of acute or chronic stress are currently being investigated.

Many membrane associated receptors have been shown to be sensitive to alterations in their lipid milieu. Changes in membrane lipids induced by activation of phospholipase A_2 (PLA $_2$) (an endogenous constituent of membranes) has been proposed as a physiologic mechanism for regulating receptor function. We have shown a differential sensitivity of "peripheral" and "central" benzodiazepine receptors to this enzyme. Phospholipase A_2 slightly increased the apparent affinity of the central benzodiazepine receptor ligands [^3H]flunitrazepam and [^3H]3-carboethoxy-B-carboline, with no concomitant change in the B_{max} of these ligands. In contrast, GABA enhanced [^3H]flunitrazepam was unaffected by PLA $_2$. Both pyrazolopyridine and barbiturate enhanced [^3H]flunitrazepam binding were, however, reduced by very low (0.002 U/ml) concentrations of PLA $_2$. Since both pyrazolopyridines and barbiturates bind to sites at or near the chloride ionophore, we examined the effects of PLA $_2$ on the specific chloride ionophore ligand [^3S]butylbicyclophosphorothionate (TBPS). It was found that PLA $_2$ inhibited [^3S]TBPS binding at the same concentrations needed to disrupt barbiturate and pyrazolopyridine enhanced [^3H]flunitrazepam binding. The inhibition of [^3S]TBPS binding by PLA $_2$ was manifest as a reduction in the B_{max} of this ligand with no change in the apparent affinity. PLA $_2$ was also found to reduce the apparent affinity of [^3H]Ro5-4864 to "peripheral" benzodiazepine receptors and the reduction in the apparent affinity of [^3H]Ro5-4864 was

independent of the tissue source (e.g. the same reduction in apparent affinity was found in heart, kidney and brain membranes).

On screening extracts of various tissues for inhibitors of [³H]Ro5-4864 binding we isolated and purified a 14 kDa protein "antralin" which was subsequently shown to have PLA₂ activity. Recently, "antralin" has been purified to homogeneity and sequenced using automative Edmann analysis of tryptic fragments. Antralin was shown to be a member of the PLA₂ family based on its amino acid sequence. Substrate and Ca⁺⁺ dependency supported its PLA₂-like activity. Antibodies raised against antralin also cross-react with purified porcine pancreatic PLA₂. Studies on the possible physiological role of this protein in peripheral organs (stomach) and brain are currently underway.

Recently, the nucleotide sequence (along with the deduced amino acid sequence) of both the alpha and beta subunits of the bovine GABA_A receptor have been established through classic cloning techniques. Using this sequence we synthesized several oligonucleotide probes for both the alpha and beta subunit and screened a GT₁₁ cDNA library prepared from human brain. Several near full length cDNA clones for the alpha subunit have been isolated and have been used as probes to study the expression of receptor protein during or following various pharmacological and environmental conditions.

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12. Paul SM, Crawley JN, Skolnick P: The neurobiology of anxiety: the role of the GABA/benzodiazepine receptor complex. In: American Handbook of Psychiatry, in press.
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14. Schwartz RD, Seale TW, Skolnick P, Paul SM: Differential seizure sensitivities to picrotoxinin in two inbred strains of mice (DBA/2J and BALB/c ByJ): Parallel changes in GABA receptor-mediated chloride flux and receptor binding. J Neurochem, in press.
15. Schwartz RD, Wess MJ, Labarca R, Skolnick P, Paul SM: Acute stress enhances the activity of the GABA receptor-gated chloride ion channel in brain. Brain Res 1987;:411:151-5.
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17. Skolnick P, Paul SM: Benzodiazepines and nonbenzodiazepines. In: O'Brien RA (ed), Receptor Binding in Drug Research. New York, Marcel Dekker, Inc., 1987, pp 53-75.
18. Suzdak PD, Glowa JR, Crawley JN, Schwartz RD, Skolnick P, Paul SM: A selective imidazobenzodiazepine antagonist of ethanol in the rat. Science 1986;234:1243-7.

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20. Suzdak PD, Paul SM, Crawley JN: Effects of Ro15-4513 and other benzodiazepine receptor inverse agonists on alcohol-induced intoxication in the rat. J Pharmacol Exp Ther, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02186-05 NS
PERIOD COVERED October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Brain Recognition Sites for Stimulants and Antidepressants: Relationship to Pharmacological Activity		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	S.M. Paul	Chief NS, NIMH
Other:	J.N. Crawley	Senior Staff Fellow NS, NIMH
	E. Lestringant	Visiting Associate NS, NIMH
	G. Muscettola	Visiting Scientist NS, NIMH
	P. Skolnick	Pharmacologist LBC, NIADDK
COOPERATING UNITS (if any) Laboratory of Bioorganic Chemistry, NIADDK; Section on Molecular Pharmacology, NS, NIMH		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Section on Preclinical Studies		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.5	0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Recognition sites for a variety of <u>psychotherapeutic drugs</u> have been identified in the central nervous system. Over the past several years we have attempted to identify recognition sites for other common psychotropic drugs including tricyclic antidepressants and the <u>psychomotor stimulants, amphetamine and methylphenidate</u> . In each case saturable, and stereospecific binding sites have been delineated; and for amphetamine and methylphenidate relatively good correlations have been observed between the affinities of a series of analogues <u>in vitro</u> and at least some of the pharmacological properties of these agents. Recent work has shown that the [3H] (+)-amphetamine binding site in hypothalamic membranes is sensitive to circulating levels of blood glucose. Hypoglycemia decreases, and hyperglycemia increases, the number of [3H](+)-amphetamine binding sites in hypothalamic membranes respectively. Furthermore, these changes seemed to be coupled to the activity of (Na+ K+) (ATPase; and there is a good correlation between the changes in [3H](+)-amphetamine and [3H]-ouabain binding both <u>in vivo</u> and <u>in vitro</u> . More recent studies have shown that [3H]-mazindol a chemically unrelated anorectic/psychostimulant also can be used to label the [3H] (+)-amphetamine cognition site and that there is a good correlation between the inhibition of [3H]-mazindol binding by a series of phenylethylamines and their anorectic activities in rats. These data suggest the existence of a membrane-bound receptor complex capable of "sensing" circulating glucose concentration and in regulating both glucostatic ingestive behavior and perhaps some aspects of the central regulation of energy metabolism. Recent work has demonstrated that genetically obese mice (ob/ob) have an abnormality in this system and fail to respond to glucoprivic feeding signals. Over the past year, we have developed a method for measuring ouabain-sensitive ⁸⁶ Rb uptake into synaptoneurosome and have used this method to measure "sodium pump" activity after treatment with anorectic drugs and in genetically obese rodents.		

Project Description:Objectives:

1. To elucidate the mechanisms of action of important psychotropic drugs such as the psychomotor stimulants, anorectic agents and antidepressants.
2. To understand the neurochemical change associated with animal models of hyperphagia and obesity.

Methods Employed:

(See: 1984 Annual Report, pp 707-712 Project Number Z01 MH 02816-02 NS, Brain Recognition Sites for Stimulants and Antidepressants: Relationship to Pharmacological Activity.)

Major Findings:

High affinity, stereospecific binding sites for [3H] mazindol have been previously described in rodent brain. The highest density of these sites are found in the synaptosomal fraction of brainstem and hypothalamus. A good correlation has been demonstrated between the ability of a series of phenylethylamine derivatives in displacing [3H] mazindol binding and their potencies as anorectic agents. These observations suggest that the [3H]-mazindol binding site (anorectic drug recognition site, ADRS) may be involved in the appetite suppressant actions of chemically unrelated anorectic agents. The ADRS has also been studied in genetically obese mice (ob/ob). In these animals the density of hypothalamic ADRS is greater than in lean litter mates. Furthermore, food deprivation of rats (24-72 hours) results in a dramatic (35-50%) reduction in the density of hypothalamic ADRS. Refeeding food-deprived animals for a four hour period (or allowing access to a 10% glucose solution) results in a return of ADRS density to control values. These data suggest that the ADRS may be intimately involved in the regulation of feeding behavior. In more recent experiments the changes in ADRS during food deprivation and refeeding have been localized to discrete hypothalamic and brainstem nuclei (paraventricular nucleus and nucleus tractus solitarius).

Since the ADRS in hypothalamus is decreased following 24 hours of food deprivation (30% reduction in Bmax), and the site density restored to control levels if the animals are permitted to refeed for four hours we have examined the factors responsible for this rapid modulation in site number. Good correlations between circulating glucose concentration and the changes in ADRS during food deprivation and refeeding suggest that glucose (or a metabolite) regulate the density of ADRS.

Injection of 2-deoxy-D-glucose also elicits a significant increase in ADRS in the hypothalamus and brainstem of rats and mice. However, this treatment did not alter ADRS in other brain regions. Further, injection of 2-deoxy-D-glucose elicited increases in ADRS density that were again confined to the PVN and NTS. Interestingly, if animals are permitted access to food during the four hour

interval following injection of 2-deoxy-D-glucose, no increase in ADRS is observed. These observations suggest that the ADRS in hypothalamus are coupled to glucose utilization and "hunger". The good correlation previously reported between the ability of a number of phenethylamines to inhibit ADRS and their potencies as anorectics may thus link the anorectic actions of phenethylamines with their ability to effect glucose-responsive neurons in the hypothalamus. Although the density of ADRS in hypothalamus is increased in genetically obese (ob/ob) mice the latter fail to increase food intake following administration of 2-deoxy-D-glucose. Further no further increase in hypothalamic ADRS is observed in ob/ob mice following 2-deoxy-D-glucose administration.

In related experiments the regulation of ADRS in hypothalamic tissue slices *in vitro* have confirmed that glucose plays a major role in determining the density of ADRS. Moreover, the ability of glucose to stimulate ADRS in hypothalamic slices *in vitro* is blocked by ouabain and correlated with similar increases in [3H]ouabain binding and Na+K+ ATPase activity. These data suggest a close functional coupling between the ADRS and Na+K+ ATPase. In related studies we have found that the genetically obese mouse has not only an increased density of ADRS, but of [3H]ouabain binding and Na ATPase activity in several brain regions. Together, with the lack of hyperphagic response to 2-deoxy-D-glucose, these data suggest that the genetically obese mouse has an altered glucostatic satiety signal.

Previous studies in our laboratory have demonstrated the presence of high affinity, stereospecific binding sites for [3H] (+) threo-methylphenidate in the striatum and brainstem of the rat. Subsequent studies demonstrated that the binding of [3H]-methylphenidate is localized to synaptosomes, and that the binding is dependent on the presence of sodium. Intraventricular administration of 6-hydroxydopamine or medial forebrain bundle lesions results in a significant loss of [3H]-methylphenidate binding in striatum which is highly correlated with a loss in the capacity of this tissue to take up [3H]-dopamine. Structure-activity studies suggest that this site is associated with a dopamine transport system since a good correlation ($r=0.88$, $p<.001$) was found between the potencies of a series of compounds to inhibit [3H]-dopamine uptake and [3H]-methylphenidate binding. These findings suggest that the methylphenidate binding site may be part of a dopamine "transporter". In a related series of experiments several diphenyl-substituted piperazines (GBR-12935, GBR-12921) have been tested for their selectivity in inhibiting dopamine uptake. The marked specificity of these compounds in inhibiting dopamine uptake has prompted the radioactive labeling of GBR-12935. [3H]-GBR-12935 appears to be a "super high affinity" ligand for the dopamine uptake site and may be useful for *in vivo* imaging of dopamine-containing neurons. Studies with postmortem human brain have further documented the association of [3H] GBR-12935 binding to dopamine neurons since we observed significant decreases in [3H] GBR-12935 binding in striatal tissue from Parkinson's patients.

Significance to Biomedical Research and Program of the Institute:

All of the drugs under investigation have important psychotropic and behavioral actions and are either of therapeutic benefit or reliably mimic various

behavioral states. Thus, an understanding of their mechanisms of actions should be of value to understanding the behavioral and psychopathological states responsive to treatment with these agents.

Proposed Course:

Studies will continue on the various recognition sites described above to more fully elucidate their pharmacological as well as physiological significance. A major emphasis will be placed on defining the alterations in ADRS density that occur *in vivo* during various manipulations of "appetite" and "satiety", in order to test the hypothesis that these sites are coupled to a physiological mechanism regulating food intake (particularly carbohydrate intake) in animals. The relationship between ADRS and the neuronal form of Na⁺K⁺ ATPase will also be investigated using both binding, enzyme assay, and ⁸⁶Rb flux measurements. Emphasis will be placed on whether these binding sites label some novel postsynaptic effector system and whether conventional neurotransmitters such as dopamine and serotonin regulate these sites *in vitro*.

Publications

1. Hauger, R.L., Skolnick, P. and Paul, S.M.: Brain recognition sites for typical and atypical antidepressants. In: Advances in Human Psychopharmacology, Vol. IV, G.D. Burrows and J.S. Werry (Eds.), Pergamon Press, New York, in press.
2. Angel, I, Kiss, A., Stivers, J.A., Skirboll, L. Crawley, J.N. and Paul, S.M.: Regulation of [3H] Mazindol binding to subhypothalamic areas: involvement in glucoprivic feeding. Brain Res. Bull., Vol. 17, 873-877, 1986.
3. Angel, I., Luu, M.D. and Paul, S.M.: Characterization of [3H] mazindol binding in rat brain: sodium-sensitive binding correlates with the anorectic otencies of phenylethylamines. J. Neurochem., Vol. 48, 491-497, 1987.
4. Janowsky, A., Vocci, F., Berger, P., Angel, I., Zelnik, N., Kleinman, J.E., Skolnick, P. and Paul, S.M.: [3H] GBR-12935 Binding to the dopamine transporter is decreased in the caudate nucleus in Parkinson's Disease. J. Neurochem., Vol. 29, 617-621, 1987.
5. Labarca, R., Janowsky, A. and Paul, S.M.: Neurotransmitter-Stimulated inositol phosphate accumulation in hippocampal slices. In: Conn PM and Means AR (eds), Methods in Enzymology, Vol 141. Orlando, Academic Press, pp192-201, 1987.
6. Skolnick, P., Schweri, M.M., Rafferty, M.F., Rice, K.C., Janowsky, A.J. and Paul, S.M. [3H]-threo-(±)-Methylphenidate binding in neuronal dopamine uptake sites in corpus striatum: correlation with the stimulant properties of ritalinic acid esters. J. Neurochem., in press.
7. Hauger, R.L., Rehavi, M., Angel, I., Janowsky, A., Skolnick, P. and Paul, S.M. Receptor-mediated mechanisms of antidepressant drug action. Forthcoming in Psychiatry, Vol 3, Section 2: Psychobiological Foundations of Clinical Psychiatry

(L.J. Judd and P.M. Groves, section editors), J.B. Lippincott Company,
Philadelphia, PA, 1987

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02340-02 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical and Clinical Studies of Gaucher Disease and Other Neurogenetic Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E.I. Ginns, Head, Molecular Neurogenetics Unit, NS, NIMH
 Others: W. Eliason Guest Researcher NS, NIMH
 M. LaMarca Guest Researcher NS, NIMH
 B. Martin Visiting Scientist NS, NIMH
 B. Martin Guest Researcher NS, NIMH
 K. Maysak Guest Researcher NS, NIMH
 B. Stubblefield Biologist NS, NIMH
 S. Winfield Microbiologist NS, NIMH

COOPERATING UNITS (if any)

Human Genetics Branch, National Institute of Child Health and Human Development; Interinstitute Genetics Program, NIH; Arthritis Service, Hospital for Joint Diseases, New York, NY

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Molecular Neurogenetics Unit, Preclinical Studies Section

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.6

PROFESSIONAL:

0.7

OTHER:

1.9

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

The clinical study of neurogenetic diseases provides the foundation for the development of techniques for improved diagnosis and strategies for therapy. This goal is greatly facilitated by having a comprehensive knowledge of the biochemistry and clinical heterogeneity of the disorder. Gaucher disease, the most common sphingolipidosis, has a high priority as a model for gaining insight into this group of neurogenetic disorders because of the occurrence of both neuronopathic and non-neuronopathic phenotypes as well as the broad spectrum of clinical diversity within the major types of the disorder. Once the pathophysiologic mechanisms of systemic involvement are understood, the therapy of nervous system dysfunction may be more rationally approached. Basic research on glucocerebrosidase, the enzyme deficient in Gaucher disease, has generated a more detailed understanding of the structure, biosynthesis, intracellular routing, and turnover of the enzyme. These studies will complement other studies within our branch focusing on the investigation of the potential and efficacy of gene transfer as a therapeutic approach. The use of recombinant DNA technologies to produce large amounts of both normal and mutant human proteins is being pursued.

OTHERS:

S. Tsuji	Visiting Fellow	NS, NIMH
Various	Genetic Fellows	IGP, NIH
J. Sidbury	Chief, Section on Human Biochem Genetics	HGB, NICHD
S. Stuchin	Orthopedic Hospital, Hospital for Joint Diseases, NY	

PROJECT DESCRIPTION

Biochemical: From our progress in the study of the molecular biology of Gaucher disease we plan to: purify mutant enzymes; characterize the primary amino acid sequences and post-translational processing of the mutant enzymes; correlate the structural mutations of the protein with the observed clinical heterogeneity; characterize the structure, organization, and regulation of expression of the normal mutant glucocerebrosidase genes; investigate the production of large quantities of protein using recombinant DNA methodologies; develop diagnostically useful recombinant DNA tests (i.e., RFLPs); and evaluate the potential of somatic cell gene therapy for Gaucher disease.

Clinical: Using Gaucher disease as a prototype of inherited disorders having both neurologic and non-neurologic phenotypes, clinical evaluations are undertaken in an attempt to correlate the clinical heterogeneity with both biochemical and genetic data. The role of the macrophage in pathogenesis of the numerous clinical manifestations of this disorder will be studied. Specifically, approaches to therapeutic intervention for Gaucher disease focus on the hypothesis that this disorder is macrophage-mediated. The involvement of hematopoietic derived cells in the pathogenesis of this disorder is crucial to the applicability of somatic cell gene therapy as a potential therapeutic strategy.

MAJOR FINDINGS

1. Recombinant normal glucocerebrosidase was purified on a pilot scale.
2. Rabbit polyclonal and mouse monoclonal antibodies were used for studying the glycosylation of recombinant glucocerebrosidase using Western blot analysis.
3. Amino acid sequencing was performed on recombinant glucocerebrosidase using protein immobilized on teflon membranes.
4. The post-translation processing of glucocerebrosidase in Type 1, 2 and 3 Gaucher disease was further characterized.
5. The normal glucocerebrosidase gene was sequenced.
6. The pseudogene for glucocerebrosidase was sequenced.
7. All the exons splice junctions and flanking regions of a genomic clone from a type 1 patient were sequenced and a single base change occurring in high frequency among non-neuronopathic phenotype (type 1) of Gaucher disease was identified.

8. A useful diagnostic test based on hybridization of an oligonucleotide probe, for the identification of the mutation in type 1 phenotypes of Gaucher disease was developed.
9. The expression by DNA mediated gene transfer of active human glucocerebrosidase in heterologous mammalian host cell lines was extended to include high-level production in the baculovirus expression system.
10. The correction of the enzyme deficiency in type 2 Gaucher fibroblasts in culture by retroviral mediated gene transfer was accomplished with cDNA retroviral constructs. Experiments with genomic constructs were begun.
11. The spectrum of symptoms of patients having Gaucher disease was further studied and correlated with RFLP analysis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

Gaucher disease is useful as a prototype disorder for furthering our understanding of the mechanisms responsible for the clinical heterogeneity seen within many of the neurogenetic disorders. It is the most common sphingolipidoses and many patients could benefit from the development of an efficacious therapy. The techniques and information obtained from the study of the protein processing and gene in Gaucher disease should be useful and helpful to formulating strategies for understanding the biochemical and genetic bases of other neuropsychiatric disorders.

PROPOSED COURSE

Patients having type 1, 2 or 3 Gaucher disease will be studied to further define the biochemical and genetic mechanisms responsible for the clinical heterogeneity within this disorder. The involvement of hematopoietic stem cell derived macrophages in the pathogenesis of symptoms makes type 1 Gaucher disease an attractive candidate for somatic cell gene therapy.

PUBLICATIONS

Barranger JA and Ginns EI: Glucosylceramide lipidoses: Gaucher disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds), The Metabolic Basis of Inherited Disease. New York, McGraw-Hill (in press).

Ginns EI: Molecular biology of inherited metabolic disorders. In: Handbook of Immunoblotting. CRC Press (in press).

Tsuji S, Choudary PV, Martin BM, Barranger JA, Stubblefield BK, Mayor JA, Ginns EI: A mutation in the human glucocerebrosidase gene in neuronopathic Gaucher disease. N Engl J Med 361:570-575, 1987.

van Dongen JM, Willemsen R, Ginns EI, Sips HJ, Tager JM, Barranger JA, Reuser AJJ: Subcellular localization of soluble and membrane-bound lysosomal enzymes in I-cell fibroblasts: A comparative immunocytochemical study. Eur J Cell Biol (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02341-02 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Correction of Inherited Enzyme Deficiencies by Gene Transfer

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E.I. Ginns, Head, Molecular Neurogenetics Unit, NS, NIMH

Others:	B. Martin	Visiting Scientist	NS, NIMH
	S. Tsuji	Visiting Fellow	NS, NIMH
	B. Stubblefield	Biologist	NS, NIMH
	M. LaMarca	Guest Researcher	NS, NIMH
	S. Winfield	Microbiologist	NS, NIMH
	B. Martin	Guest Researcher	NS, NIMH
	W. Eliason	Guest Researcher	NS, NIMH

COOPERATING UNITS (if any)

Center for Cancer Research, MIT and Whitehead Institute for Biomedical Research, Boston, MA

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Molecular Neurogenetics Unit, Preclinical Studies Section

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.8

PROFESSIONAL:

0.7

OTHER:

1.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The isolation and characterization of proteins involved in the pathogenesis of neurogenetic disorders has permitted the isolation of cDNA and genomic DNA that can be used to investigate the correction of inherited enzyme deficiencies using recombinant DNA techniques, specifically somatic cell gene transfer. Particularly suited for initial attempts at gene therapy are those disorders (such as Gaucher disease, the most common sphingolipidosis) in which the manifestations of the disorder are due to abnormalities of hematopoietic cells, in this case, the macrophage. In this instance the transfer of normal genes to bone marrow progenitor cells is a rationale therapeutic approach. Using the lysosomal disorder Gaucher disease as a model, we have been successful in utilizing retroviral vectors to transfer and express human glucocerebrosidase in host mouse and Gaucher cell lines. The complete correction of glucocerebrosidase activity in Type 2 Gaucher fibroblasts in culture has provided the impetus for evaluation of retroviral mediated somatic cell gene transfer of the glucocerebrosidase gene into mice by bone marrow transplantation. The initial goal of this research is the application of these recombinant DNA therapeutic strategies to the non-neuronopathic phenotypes. When our understanding of the pathogenetic mechanisms of inherited neurological and psychiatric diseases improves and when retroviral-mediated expression of genes in specific tissues and cells become more predictable, we can begin to investigate the potential usefulness of gene therapy for treatment of selected nervous system disorders.

OTHERS:

- K. Maysak Guest Researcher NS, NIMH
 R. Mulligan Center for Cancer Research, MIT and Whitehead Institute
 for Biomedical Research, Boston, MA
 B. Guild Center for Cancer Research, MIT and Whitehead Institute
 for Biomedical Research, Boston, MA
 E. Dzierzak Center for Cancer Research MIT and Whitehead Institute
 for Biomedical Research, Boston, MA

PROJECT DESCRIPTION

The isolation of cDNA and genomic DNA encoding specific proteins involved in neurogenetic disorders permits the application of recombinant DNA technologies as therapeutic approaches to the correction of these inherited enzyme deficiencies. Using the lysosomal storage disorder Gaucher disease as a prototype we are investigating the efficacy of retroviral mediated gene transfer, first applied in tissue culture and then in small animals.

MAJOR FINDINGS

1. Complementary DNA and genomic DNA clones encoding human glucocerebrosidase have been isolated and sequenced.
2. Clones for both the functional genomic DNA and a pseudogene for human glucocerebrosidase have been isolated.
3. Active human glucocerebrosidase has been transferred to both mouse and monkey cell lines using eukaryotic shuttle vectors.
4. The enzyme deficiency in a Type 2 Gaucher cell line has been corrected by transfer of the normal human glucocerebrosidase cDNA to these cells in culture.
5. Recombinant retrovirus containing human glucocerebrosidase cDNA has been used to infect mouse bone marrow cells and obtain reconstituted mice have provirus in their blood cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

The isolation of the cDNA and genomic DNA for human glucocerebrosidase and the transfer of this gene by retroviral vectors suggests that such recombinant DNA approaches may be useful therapeutic strategies for Gaucher disease and other selected genetic disorders.

PROPOSED COURSE

The project initially focuses on the DNA mediated-transfer of normal, human glucocerebrosidase to rodent and human cell lines in culture. Once the efficacy of gene transfer (particularly retroviral-mediated) is demonstrated, the approach

will be applied to appropriate human subjects with specific inherited disorders (such as Gaucher disease).

PUBLICATIONS

Choudary PV, Tsuji S, Martin BM, Guild BC, Mulligan RC, Murray GJ, Barranger JA, Ginns EI: The molecular biology of Gaucher disease and potential for gene therapy. Cold Spring Harbor Symposia 51 on Molecular Biology of Homosapiens, 1986, pp 1047-1052.

Martin BM, Tsuji S, LaMarca ME, Maysak K, Eliason W, Ginns EI: Molecular biology of Gaucher disease: Therapeutic strategies utilizing recombinant DNA technologies, in NATO Advanced Research Workshop and Inserm Symposium: Lipid Storage Disorders (Biological and Medical Aspects). Toulouse, 1987, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02342-02 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.)

Gene Regulation within the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E.I. Ginns, Head, Molecular Neurogenetics Unit, NS, NIMH

Others: B. Martin	Visiting Scientist	NS, NIMH
S. Tsuji	Visiting Fellow	NS, NIMH
S. Winfield	Microbiologist	NS, NIMH
P. Marangos	Unit on Neurochemistry	BPB, NIMH
D. Schmeckel	Neurology Department	VAMC
J. Polak	Royal Post-Graduate Medical School, London	
J. Hozier	Medical Genetics	FIT

COOPERATING UNITS (if any)

Unit on Neurochemistry, Biological Psychiatry Branch, NIMH; Veterans Administration Medical Center, Durham, NC; Royal Post-Graduate Medical School, London, England; Florida Institute of Technology, Melbourne, FL

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Molecular Neurogenetics Unit, Preclinical Studies Section

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.4

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We approached the cell-specific and developmentally regulated expression of proteins within the nervous system using the neuron specific (NSE) and non-neuronal (NNE) enolase isozymes as a model. Human brain cDNA and genomic DNA libraries were constructed so that the genes for these and other brain specific proteins could be isolated and characterized. Using both antibodies and oligonucleotide probes, cDNAs for both human NSE and NNE have been isolated and sequenced. Employing unique regions of these cDNA clones as probes, the developmentally and cell-specific regulated appearance of mRNA for each of these proteins can be investigated using in-situ hybridization. The human chromosome loci for each of these isozymes will be identified. In addition, the isolation of human genomic clones for each of these proteins should provide information on the regulation of expression of neuron and glial specific proteins during cell differentiation of the human nervous system in normal and disease states. The normal specificity of NSE for neural derived cell lines and the availability of specific DNA probes for NSE should provide a useful approach to the characterization of neural derived normal and tumor cell lineages.

PROJECT DESCRIPTION

The enolase isozymes will be used as a model for studying the transcriptional and translational control of developmentally regulated genes within the human nervous system. The genomic organization and regulation of expression of these genes will be investigated.

MAJOR FINDINGS

1. Human brain cDNA and genomic DNA libraries have been constructed.
2. Complementary DNA clones for human neuron specific and non-neuronal enolases have been isolated and sequenced.
3. Specific cDNA probes for NSE and NNE have been demonstrated to be useful for high resolution in-situ chromosome localization and tissue hybridization studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

A description of the transcriptional and translational control mechanisms for neuron specific and non-neuronal enolases should provide a more detailed understanding of the mechanisms involved in regulation of gene expression in neurons and glia within the human nervous system during development and differentiation.

PROPOSED COURSE

The project will focus on the description of the genomic organization and control mechanisms of expression of NSE and NNE genes. The involvement of mutations in the genes for these proteins in the pathogenesis of neuropsychiatric disorders will be investigated.

PUBLICATION

Schmechel DE, Marangos PJ, Martin BM, Winfield S, Burkhart DS, Roses AD, Ginns EI: Localization of neuron-specific enolase (NSE) mRNA in human brain. Neurosci Lett 76:233-238, 1987.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02343-02 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Inherited Neurologic and Psychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E.I. Ginns, Head, Molecular Neurogenetics Unit, NS, NIMH

Others: S.M. Paul	Chief, Clinical Neuroscience Branch	NS, NIMH
D. Pickar	Chief, Section on Clinical Studies	NS, NIMH
J. Kelsoe	Medical Staff Fellow	NS, NIMH
B. Martin	Visiting Scientist	NS, NIMH
B. Stubblefield	Biologist	NS, NIMH
S. Winfield	Microbiologist	NS, NIMH

COOPERATING UNITS (if any)

Pediatrics Department, Johns Hopkins School of Medicine, Baltimore, MD; Florida Institute of Technology, Melbourne, FL; Dept of Anatomy & Neurobiology, Washington Univ School of Medicine

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Molecular Neurogenetics Unit, Preclinical Studies Section

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.8

PROFESSIONAL:

0.9

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We approached the characterization of the mutations responsible for inherited neurological or psychiatric disorders by studying the gene organization of specific proteins that might have a role in the pathogenesis of the clinical manifestations. Using the inherited lysosomal storage disorders, Gaucher disease and Fabry disease, as models, we demonstrated that the phenotypic heterogeneity seen within these inherited disorders is a consequence of different mutations, each affecting protein activity and influencing the processing, compartmentalization and/or stability of the protein. Similar approaches are being used to investigate the involvement of genes on chromosome 11 in mania-depression and other psychiatric disorders. Recombinant DNA techniques have been used to elucidate the structure of the gene for the proteins (enzymes and receptors) involved in these and other neuropsychiatric disorders. Restriction fragment length polymorphisms (RFLPs) have been identified that are useful for the identification of mutations in Gaucher disease that frequently occur in both non-neuronopathic and neuronopathic phenotypes. Northern blot analysis provides further details of the structure of the normal and mutant genes. The molecular mechanisms leading to nervous system involvement in these disorders have also been investigated. The results of this research should provide a more rational foundation for the diagnosis and formulation of therapeutic strategies for these inherited disorders. Genes on chromosome 11 specific for neurotransmitter biosynthesis have been isolated (for example, human tyrosine hydroxylase and tryptophan hydroxylase). Comparison of normal gene sequence to the gene sequence in Amish manic-depressive patients is in progress. Recombinant DNA approaches have been used to produce large amounts of tyrosine hydroxylase isozyme for structural and biochemical studies.

OTHERS

B. Migeon Pediatrics Department
 J. Hozier Medical Genetics
 K. O'Malley Anatomy & Neurobiology, Washington Univ School Med

Johns Hopkins
 FIT

PROJECT DESCRIPTION

The diversity in the presentation of neurogenetic disorders may be a consequence of multiple allelic mutations. The understanding of the mechanisms of this phenotypic heterogeneity will be derived from genetic and biochemical analyses. Recombinant DNA techniques are used to isolate and characterize the genes for specific proteins. The study of mutations should elucidate the structural abnormalities and consequences of the abnormal biosynthesis and post-translational processing of the mutant proteins. The identification of RFLPs associated with clinical manifestations is studied using cDNA and genomic DNA probes. The comparison of gene expression for proteins in neural and non-neural tissues should extend our understanding of protein regulation.

MAJOR FINDINGS

1. Complementary DNA and genomic libraries were constructed from human brain tissue.
2. Human cDNA clones encoding active tyrosine hydroxylase have been isolated and sequenced.
3. A single base mutation has been identified within the coding regions of a type 1 (non-neuronopathic) Gaucher genomic DNA clone. This mutation occurs in high frequency in patients having non-neuronopathic Gaucher disease.
4. Chromosome 11 specific probes (globin, harvey ras, tyrosine hydroxylase) have been obtained for RFLP analysis of patient's having bipolar affective illness.
5. The normal gene for human tyrosine hydroxylase was isolated and sequenced.
6. Active recombinant human tyrosine hydroxylase has been produced.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

The description of the molecular basis for neuropsychiatric disorders should provide a more rational basis for the development of diagnostic and therapeutic strategies.

PROPOSED COURSE

The project will focus on the biochemistry and genetics of these disorders to obtain a more complete understanding of the mechanisms responsible for the

clinical manifestations of these inherited disorders. This information will be used to develop diagnostic and therapeutic strategies.

PUBLICATIONS

Willemsen R, Van Dongen JM, Ginns EI, Sips HJ, Schram AW, Tager JM, Barranger JA, Reuser AJJ: Ultrastructural localization of glucocerebrosidase in cultured Gaucher's disease fibroblasts by immunocytochemistry. J Neurol 234:44-51, 1986.

Ginns EI: Application of immunoblotting to the study of the molecular biology of inherited metabolic disorders. In: Handbook of Immunoblotting (Chap 9.5). CRC Press (in press).

Jonsseon LMV, Murray GJ, Sorrell S, Strijland A, Aerts JFGM, Ginns EI, Barranger JA, Tager JM, Schram AW: Biosynthesis and maturation of glucocerebrosidase in Gaucher fibroblasts. Eur J Biochem 164:171-179, 1987.

O'Malley KL, Anhalt M, Martin BM, Kelsoe JR, Winfield SL, Ginns EI: Isolation and characterization of the human tyrosine hydroxylase gene: Identification of 5' alternative splice sites responsible for multiple mRNAs. Biochemistry (in press).

Tsuji S, Martin BM, Kaslow D, Migeon BR, Choudary PV, Stubblefield BK, Mayor JQ, Murray GJ, Barranger JA, Ginns EI: Signal sequence and DNA mediated expression of human lysosomal alpha-galactosidase A. Eur J Biochem 165:275-280, 1987.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02344-02 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropsychiatric Disorders: Protein Structure-Activity Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: B. Martin, Visiting Scientist, NS, NIMH

Others: E. I. Ginns Head, Molecular Neurogenetics Unit

NS, NIMH

W. Eliason Guest Researcher

NS, NIMH

K. Maysak Guest Researcher

NS, NIMH

L. Possani Free University of Mexico, Mexico

COOPERATING UNITS (if any)

Free University of Mexico, Mexico

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Molecular Neurogenetics Unit, Preclinical Studies Section

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.6

PROFESSIONAL:

0.5

OTHER:

1.1

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research is part of an effort to better understand the molecular mechanisms underlying human nervous system development and function, as well as the pathogenesis of certain neurogenetic disorders. Our studies have focused on structural and active site properties of the human non-neuronal and neuron specific enolases, lysosomal hydrolase (glucocerebrosidase and alpha-galactosidase A), other enzymes (particularly those peptides and proteins that interact with excitable membranes), and venom toxins. Proteins are purified from both human and animal tissues using affinity chromatography, electrophoretic separation, and high performance liquid chromatography. Using microsequencing techniques, the complete amino acid sequence of glucocerebrosidase, antralin and major portions of sequences for the neuronal and non-neuronal enolases, venom toxins, and alpha-galactosidase A have been obtained. Peptide maps of both normal and mutant proteins are generated using chemical (cyanogen bromide) and enzymatic (trypsin, thermolysin, V8 protease) cleavage. The identification of carbohydrate attachment sites, sulphydryl residues, and intra-chain disulfide residues is used to predict protein structure. Alkylating agents and enzyme inhibitors are used to define active sites. From the primary protein sequence, hydrophobic and hydrophilic domains of the protein are identified.

Information obtained from these protein structure studies permits the design of oligonucleotides and peptides that are synthesized for collaborative research (see Z01 MH 02343-02 NS) involving antibody production, cDNA cloning, DNA sequence analysis and in vitro mutagenesis.

PROJECT DESCRIPTION

A goal of this project includes the identification of primary and tertiary structure of the proteins. Once these aspects of protein structure are elucidated, a three-dimensional model can be constructed using the secondary structure data obtained from computer modeling. This information will be useful in defining the hydrophilic and membrane domains of the protein. Active sites are identified using sulfhydryl reagents and specific inhibitors and activators. This information is used to design synthetic oligonucleotides and peptides for collaborative research.

MAJOR FINDINGS

1. The first complete amino acid sequence of a human lysosomal enzyme (glucocerebrosidase) was determined, as well as identification of the four carbohydrate attachment sites and all three disulfide bridges in this protein.
2. Biochemical characterization, including partial amino acid sequence, of a novel calcium binding protein from snake venom.
3. Determination of the amino acid sequence of antralin, an unusual phospholipase A2 from rat stomach.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

The neuron and non-neuronal enolases are used as a model system to investigate the developmentally controlled expression of specific proteins within the nervous system. The lysosomal hydrolases are used as prototypes for understanding the phenotypic heterogeneity within the neurogenetic disorders. Studies of venom toxins should provide information on the structure of ion channels within the nervous system. Together these studies further our understanding of the structure-function relationships of nervous system proteins.

PROPOSED COURSE

The project will focus on the proteins described above as well as other proteins involved in lysosomal and neuropsychiatric disorders.

PUBLICATIONS

Alagon AC, Guzmán HS, Martin BM, Ramirez AN, Carbone E, Possani LD: Isolation and characterization of two toxins from the Mexican scorpion *Centruroides limpidus karsch*. Comp Biochem Physiol (in press).

Mussar KJ, Murray GJ, Martin BM, Viswanatha T: A rapid chromatographic assay procedure for peptide: N-glycosidase activity. J Chromat (in press).

Zasloff M, Martin BM, Chen H-C: Anti-microbial polypeptides from *X. laevis* skin: sequencing and synthesis of the Maqaini family. Proc Natl Acad Sci (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00112-10 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Endorphin Research in Mental Illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. Pickar, Chief, Section on Clinical Studies NS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

INCORPORATED UNDER PROJECTS Z01 MH 02181-05 NS AND Z01 MH 02184-05 NS IN 1987

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02181-05 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. Pickar Chief, Section on Clinical Studies NS, NIMH

Others: S.M. Paul	Chief	NS, NIMH
A.F. Breier	Medical Staff Fellow	NS, NIMH
J.R. Kelsoe	Medical Staff Fellow	NS, NIMH
P.B. Lucas	NRSA Fellow	NS, NIMH
C.N. Pato	NRSA Fellow	NS, NIMH
M.H. Rapaport	Medical Staff Fellow	NS, NIMH
J.L. Schreiber	Social Worker	NS, NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, NIMH; Neuropsychiatry Branch, St. Elizabeths Hospital, NIMH

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

7.0

PROFESSIONAL:

5.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project studies the psychobiology of schizophrenia and attempts to develop improved strategies for its treatment. An important goal is the understanding of the mechanism of action of neuroleptic drugs. We have observed that neuroleptic-induced time-dependent decrease in levels of plasma homovanillic acid (HVA), a major dopamine metabolite, correlates with antipsychotic drug response, suggesting that slow to develop changes in dopamine turnover may underlie the antipsychotic action of neuroleptics. This clinically relevant dopamine marker is further studied using a strategy in which peripherally derived HVA is reduced by the administration of debrisoquin, a MAO inhibitor which does not enter the CNS. In a double-blind, treatment trial, we have observed significant reduction in psychosis in some patients when alprazolam is added to neuroleptic treatment; the use of benzodiazepines to augment neuroleptic response is under investigation in outpatient trials. These data contrast with the negative results found when the calcium channel blocker, verapamil, was administered to neuroleptic-free schizophrenic patients. In a recently completed study using magnetic resonance imaging (MRI) performed in collaboration with the Clinical Brain Disorders Branch, NIMH, compelling evidence was gained which supports enlargement of lateral and 3rd ventricular volumes in schizophrenic patients. A follow-up study of previous inpatients from our program has recently been completed and holds promise for delineating biological correlates of outcome in schizophrenia. A controlled study of expressed emotion in families of schizophrenic patients is in progress. The proposed course involves the further development of an outpatient research program which includes controlled pharmacologic treatment studies and the evaluation of family schizophrenia pedigrees for collaboration with the Clinical Neuroscience Molecular Biology Laboratory. Collaboration with the NIMH positron emission tomography (PET) program focusing on the mechanism of action of typical and atypical neuroleptics using glucose utilization studies and the development of new PET ligands will be pursued.

OTHER PROFESSIONAL PERSONNEL

O.M. Wolkowitz	Medical Staff Fellow	NS, NIMH
R.M. Cohen	Chief, Clinical Brain Imaging Section	LPP, NIMH
D. Weinberger	Chief, Section on Clinical Neuropsychiatry	NPB, NIMH

PROJECT DESCRIPTION

This project is part of the research program of the Section on Clinical Studies of the Clinical Neuroscience Branch. This section conducts clinical research on the 4-East Nursing Unit and the ACRF of the Clinical Center.

Despite enormous research and clinical efforts to alleviate symptoms of schizophrenia, the group of drugs known as neuroleptics have remained the principal pharmacologic agents for treatment of this illness. Moreover, the effects of neuroleptics on CNS dopamine systems also represent the cornerstone for the dopamine hypothesis of schizophrenia. An important facet of our research program is the pharmacotherapy of schizophrenia, including studies of the mechanism of action of neuroleptic drugs.

We have performed longitudinal clinical studies in which neuroleptic-induced alterations in dopamine function are assessed using levels of plasma homovanillic acid (HVA). Patterns of neuroleptic-induced alteration in plasma HVA level are examined as predictors of clinical response. Administration of the MAO-A inhibitor, debrisoquin, is used to enhance the relative contribution of CNS derived HVA to levels which circulate in plasma. Positron emission tomographic (PET) studies of typical and atypical neuroleptic drugs in which regional brain glucose utilization is examined are currently in progress.

Preclinical pharmacology of dopamine neurons, including new developments in the understanding of mesocortical dopamine neuronal function, has prompted studies of the clinical effects of the triazolobenzodiazepine, alprazolam, as an additive treatment to neuroleptics in schizophrenic patients. Other aspects of our research program include efforts to identify structural abnormalities in the brains of schizophrenic patients using CT and MRI techniques as well as pathological mechanisms, e.g., autoimmune phenomena, which may underlie these structural abnormalities.

An outpatient program in schizophrenia research has recently been initiated. We have completed a follow-up study of patients who had been discharged from our research program at least two years previously. The goal of this work is to examine the course of illness and to identify predictors of outcome. Pilot outpatient investigation of neuroleptic augmenting effects of the benzodiazepines, lorazepam and alprazolam, are in progress.

METHODOLOGY

(See: 1985 Annual Report, pp 471-474, Project Number Z01 MH 02181-03 NS, Neurobiology of Schizophrenia)

We have administered the non-CNS active MAO inhibitor, debrisoquin, to reduce the non-CNS contribution of HVA to levels which circulate in plasma. This

pharmacologic strategy is intended to enable better assessment of CNS dopamine function through plasma sampling and is applied to longitudinal studies of neuroleptic treatment.

A follow-up study of schizophrenic patients who had previously participated in our 4-year research program has been performed. All patients discharged at least 2 years prior to this study were contacted for follow-up interviews. Data focused on levels of social, work and personal function, on current symptomatology and overall illness course. Data from the index hospitalization is then examined for correlation with outcome.

The effect of metabolic stress produced by the infusion of 2-deoxy-D-glucose (2-DG) (50 mg/kg) in neuroleptic-treated schizophrenic patients and controls has been performed. Behavioral and neurochemical response is studied.

A family study in which expressed emotion response to a schizophrenic family member as compared with the expressed emotion response to a non-ill sibling undergoing a stressful life period has now been completed. This study examines the specificity of the expressed emotion response seen in families of schizophrenic patients.

MAJOR FINDINGS

1. Expanded data support our initial findings that neuroleptic treatment produces time-dependent decreases in plasma levels of the dopamine metabolite, HVA, and that neuroleptic-induced changes in levels of plasma HVA correlate with clinical response. Preliminary data using the debrisoquin strategy show similar time-dependent decreases in plasma HVA levels, supporting the notion that this pattern reflects similar changes in CNS dopamine systems. Preliminary analysis of longitudinal MHPG and HVA data indicate that changes in MHPG and HVA together provide the best information regarding negative symptoms, whereas HVA alone is the best predictor of positive symptoms.

A particularly interesting aspect to this metabolite work is the contrast between CSF and plasma HVA response to neuroleptic treatment. In contrast to the neuroleptic-induced reduction seen in plasma HVA, CSF HVA levels tend to increase, and remain increased, in response to neuroleptic treatment. This discrepancy may relate to particularly prominent effects of mesocortical dopamine neurons in determining levels of CSF HVA.

2. We have demonstrated that the relative balance between positive and negative symptoms in schizophrenia are state- (e.g., drug-treated vs drug-free) rather than trait-dependent. These findings have important implications with regard to classifying schizophrenic patients into positive and negative "types."

3. We find no consistent relationship between negative and positive symptom profiles and CT abnormalities including generalized and frontal cortical atrophy nor lateral or third ventricular enlargement. We have observed that both medical and schizophrenic patients share significant enlargement in lateral ventricular size in comparison to normal controls. However, an abnormality unique to the schizophrenic patient is CT scan changes consistent with prefrontal, but not generalized, atrophy. Results from our completed MRI study provide additional

support for the notion of structural brain changes in schizophrenic patients. Specifically, enlargement of the cerebral ventricular system is found.

4. In a completed double-blind controlled investigation, we have observed that the calcium channel blocker, verapamil, is without therapeutic effect in neuroleptic-free patients. Significant increases in levels of plasma in CSF HVA were, however, observed.
5. In a completed study of twelve patients, we have observed significant improvement in psychosis ratings when alprazolam is added to a stabilized regimen of neuroleptic treatment. These clinical effects appear to be divisible into responders (5 of 12 patients) and nonresponders (5 of 12 patients; two partial responders) categories. In comparison with nonresponders, responders have greater prefrontal atrophy as seen on CT scan and greater alprazolam-induced decreases in levels of plasma HVA than nonresponders.
6. Analysis of data from the follow up study of schizophrenic patients is in progress.
7. 2-DG produces marked metabolic stress in normal subjects and in schizophrenic patients. The response is characterized by increased levels of cortisol and ACTH. Although plasma levels of HVA were significantly increased in both patients and controls, schizophrenic patients showed a significantly greater 2-DG stimulated increase in plasma HVA, despite neuroleptic treatment, than did controls.
8. The expressed emotion study is currently undergoing data analysis.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

Schizophrenia is a major public health problem in the United States. This project attempts to study possible etiologic factors in schizophrenia, to develop a better understanding of mechanisms underlying current pharmacologic treatments and to improve the overall current pharmacotherapy of schizophrenia.

Our recent findings with regard to change in levels of plasma HVA during neuroleptic treatment and their demonstrated relationship to clinical response are important to the field as they suggest the possibility that monitoring levels of plasma HVA may be useful as a marker for the antipsychotic effects of neuroleptics. This work, focusing on change in dopamine system activity during neuroleptic treatment, is being pursued further using debrisoquin administration strategies to enhance the CNS HVA "signal." The indication that the antipsychotic process may involve a step-wise process, rather than a direct relationship with dopamine receptor blockade, provides new opportunity for strategies to augment neuroleptic response.

The augmentation of antipsychotic effects by the addition of alprazolam represents a potentially important new pharmacotherapy for schizophrenia. The elucidation of the mechanism by which this clinical response occurs is an important goal for future research.

Our findings from CT studies are important as they add continued support for the notion of structural pathology in the frontal cortex of schizophrenic patients. Confirmation of ventricular enlargement by MRI is an important contribution to the field. Our inability to demonstrate the trait nature of the balance between positive and negative symptoms in schizophrenia and their lack of relationship to abnormality by CT scan raise into question the notion that structural brain abnormality is associated with specific types of schizophrenic symptoms.

New treatment strategies which develop from this work would have considerable importance to the field of psychiatry and to the estimated 2 million patients suffering from schizophrenia in the United States. Our research ward is well-suited to studying the clinical and biological affects of pharmacotherapy of schizophrenia; we have had success in longitudinal studies of levels of plasma HVA and in the demonstration of the augmentation of neuroleptic antipsychotic effects by alprazolam.

The follow-up study of schizophrenic patients holds promise for developing correlates of outcome in schizophrenia. The question of the natural course of schizophrenia is also addressed in this study.

Paradigms for studying stress response of schizophrenic patients are important because of the role of stress in the clinical course of schizophrenia.

PROPOSED COURSE

1. We will continue to study the implications of our plasma HVA data with regard to predictors of antipsychotic response in patients with schizophrenia. Using the debrisoquin technique and comparisons of plasma and CSF levels of HVA, we hope to develop a more clear understanding of neuroleptic/dopamine system interactions.
2. We will continue to assess the efficacy and mechanism of action of alprazolam as an additive treatment to neuroleptics in schizophrenia. Moreover, we will extend this work to include outpatient studies. An outpatient study addressing this issue is currently in progress.
3. The analysis of data from our follow-up study will help to orient our thinking about factors relating to the course of schizophrenia. This study has already proven the feasibility of follow up studies at the NIMH and suggests the need for continued longitudinal study.
4. Collaboration with the NIMH PET group has increased over the past year and holds promise for developing new insights into not only the pathophysiology of schizophrenia but also the mechanism of action of neuroleptic drugs.

PUBLICATIONS

Barbaccia, M.L., Costa, E., Ferrero, P., Guidotti, A., Roy, A., Sunderland, T., Pickar, D., Paul, S.M., and Goodwin, F.K.: Diazepam binding inhibitor, a brain neuropeptide present in human spinal fluid: studies in depression, schizophrenia and Alzheimer's disease. Arch. Gen. Psychiatry 43(12):1143-1147, 1986.

- Breier, A., Arora, P.K., Pickar, D., Wolkowitz, O.M., and Paul, S.M.: Metabolic stress produces rapid immunosuppression in man. Arch. Gen. Psychiatry (in press).
- Breier, A., Wolkowitz, O.M., Doran, A.R., Roy, A., Boronow, J., Hommer, D.W., and Pickar, D.: Neuroleptic responsivity of negative and positive symptoms in schizophrenia. Am. J. Psychiatry (in press).
- Cohen, R.M., Semple, W.E., Gross, M., Nordahl, T.E., DeLisi, L.E., Holcomb, H.H., King, A.C., Morihisa, J.M., and Pickar, D.: Dysfunction in a prefrontal substrate of sustained attention in schizophrenia. Life Sci. 40:2031-2039, 1987.
- Doran, A.R., Boronow, J., Weinberger, D.R., Wolkowitz, O.M., Breier, A., and Pickar, D.: Structural brain pathology in schizophrenia revisited: prefrontal cortex pathology is inversely correlated with CSF levels of homovanillic acid. Neuropsychopharmacology (in press).
- Pickar, D.: Recent perspectives on the mechanism of action of neuroleptic drugs: implications for a time-dependent model. Schizophr. Bull. (in press).
- Pickar, D.: Pharmacotherapeutic approaches to schizophrenia. In: Jimerson, D.C., Docherty, J.P. (eds), Psychopharmacology Consultation. Washington, DC, Am. Psychiatric Press, 1986, pp 71-103.
- Pickar, D., Breier, A., and Kelsoe, J.: Plasma homovanillic acid as an index of central dopaminergic activity: studies in schizophrenic patients. NY Acad. Sci. (in press).
- Pickar, D., Breier, A., Wolkowitz, O.M., and Doran, A.R.: The biochemical basis for the antipsychotic effects of neuroleptics. Etiopathogenic Hypotheses of Schizophrenia. London, MPT Press (in press).
- Pickar, D., Breier, A., Wolkowitz, O.M., and Pato, C.: Profiles of the pharmacologic response of positive and negative symptoms in schizophrenia. Psychiatrie and Psychobiologie (in press).
- Pickar, D., Roy, A., Breier, A., Doran, A., Wolkowitz, O., Colison, J., and Agren, H.: Suicide and aggression in schizophrenia: neurobiologic correlates. Ann. NY Acad. Sci. 487:189-196, 1986.
- Pickar, D., Wolkowitz, O.M., Doran, A.R., Labarca, R., Roy, A., Breier, A., and Narang, P.K.: Clinical and biochemical effects of verapamil administration to schizophrenic patients. Arch. Gen. Psychiatry 44:113-118, 1987.
- Pickar, D., Wolkowitz, O.M., Labarca, R., Doran, A.R., Breier, A., and Paul, S.M.: Biochemical alterations produced by neuroleptics in man: studies of plasma homovanillic acid in schizophrenic patients. In: Dahl, S.G., Gram, L.F., Paul, S.M., and Potter, W.Z. (eds), Clinical Pharmacology in Psychiatry. Selectivity in Psychotropic Drug Action--Promises or Problems? (Vol 3). New York, Springer-Verlag, 1986, pp 248-254.

Roy, A., Pickar, D., Doran, A., Wolkowitz, O., Gallucci, W., Chrousos, G., and Gold, P.: The corticotropin-releasing hormone stimulation test in chronic schizophrenia. Am. J. Psychiatry 143(11):1393-1397, 1986.

Roy, A., Schreiber, J., Mazonson, A., and Pickar, D.: Suicidal behavior in chronic schizophrenic patients: a follow-up study. Can. J. Psychiatry 31(8): 737-740, 1986.

Shelton, R.C., Doran, A.R., Pickar, D., and Weinberger, D.R.: Cerebral structural pathology in schizophrenia: evidence for a selective prefrontal cortical defect. Am. J. Psychiatry (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02184-05 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Depression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. Pickar, Chief, Section on Clinical Studies NS, NIMH

Others: S.M. Paul	Chief	NS, NIMH
A.F. Breier	Medical Staff Fellow	NS, NIMH
C.N. Pato	NRSA Fellow	NS, NIMH
M.H. Rapaport	Medical Staff Fellow	NS, NIMH
O.M. Wolkowitz	Medical Staff Fellow	NS, NIMH
G.A. Roy	Visiting Associate	LCS, NIAAA

COOPERATING UNITS (if any)

Laboratory of Clinical Studies, NIAAA; Laboratory of Clinical Science, Biological Psychiatry Branch and Laboratory of Neurochemistry, NIMH

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

5.0

PROFESSIONAL:

3.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this study is to investigate selected areas of the neurobiology of depression and mania. In previous studies, we have observed that abnormality of noradrenergic and HPA axis dysfunction occur together in seriously depressed patients. We have pursued the study of the role of corticosteroids in depressive illness by examining the effect of exogenous steroid administration. We have found that orally administered dexamethasone produces selective effects on catecholamine function in depressed patients; in contrast to normal controls, depressed patients, particularly those with psychotic features, showed a significant dexamethasone-induced increase in plasma MHPG and a decrease in plasma HVA. These data suggesting abnormal corticosteroid-catecholamine interactions in depression are consistent with the possibility that hypercortisolemia itself may produce or enhance some of the biochemical changes and/or behavioral signs of depression. In a double-blind study of orally administered prednisone (80 mg/day x 5 days) to normal volunteers, we have further investigated steroid effects on the central nervous system. The relationship between stress, steroids, and mood, has been pursued in an experiment in which identical amounts of escapable and inescapable aversive noise stimuli are presented to subjects. Preliminary results suggest that inescapable but not escapable "stress" produces correlated mood and neuroendocrine response. We have examined the effects of permanent separation from one or both biological parent(s) between the ages of 2 and 17 years on the development of adult psychopathology and observed that poorer quality of home life and personal adaptation subsequent to parental loss was associated with significantly higher incidence of major depression as adults and increased levels of plasma cortisol at the time of cross-sectional study. We are currently investigating the therapeutic and biochemical effects of verapamil, a calcium channel antagonist, in manic and hypomanic patients.

OTHER PROFESSIONAL PERSONNEL

W.Z. Potter	Chief, Unit on Clinical Psychopharmacology	LCS, NIMH
D. Rubinow	Chief, Unit on Peptide Studies	BPB, NIMH

PROJECT DESCRIPTION

The purpose of this project is to investigate selected aspects of the neurobiology of depression and mania. Our studies have demonstrated the association between hyperactivity of the noradrenergic system and hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis in severely depressed patients. In further studies of the HPA axis we have examined the effect of corticosteroids, themselves, on behavior and neurotransmitter function. A research project which examines the effects of "controllable" stress and "uncontrollable" stress on behavior, HPA and catecholaminergic function has been performed in normal volunteers and depressed patients. A study of the development of depressive illness in adults who had experienced parental separation during childhood has been accomplished.

METHODOLOGY (See: 1985 Annual Report, pp 475-477, Project Number Z01 MH 02184 03 NS, Neurobiology of Depression)

Additional Methods:

1. The comparative behavioral and neuroendocrine effects of controllable and uncontrollable aversive noise stress is studied in healthy volunteers, patients with depression and patients with past histories of depression but in current remission. The experiment consists of participation in two alternatively assigned test days. On the controllable stress test day, aversive loud noise is administered and can be terminated providing a simple button push sequence is learned. On the uncontrollable stress test day the button responses are independent of noise termination and the subjects are unable to stop the noise. The amount of noise stress is held constant in both conditions by "yoking" uncontrollable noise duration to controllable noise duration. Following the noise stress, subjects are presented a mental task consisting of solving simple anagrams under time pressure. Plasma levels of cortisol and ACTH catecholamine are measured throughout the experiment. Modifications of this protocol which include mild electric shock in place of noise stress have been utilized in order to enhance the stress response.
2. The effect of early parental loss on the development of adult psychopathology was examined in a cohort of self-selected patients who had experienced permanent separation from one parent between the years of 2 and 17 years of age. SADS-RDC life time diagnoses were determined for all subject. Environmental factors relating to personal and family adaptation to loss, family history and neuroendocrine assessment at follow up were examined for relationship for the development of adult psychopathology.
3. A prospective investigation of the clinical and therapeutic effects of the calcium channel antagonist, verapamil, in patients with manic and hypomanic symptoms is currently being performed on the 4-East Nursing Unit. Methodology is the same as that already utilized to study the effects of verapamil in

schizophrenic patients, and include longitudinal plasma amine metabolite sampling and CSF assessment of neurotransmitter level.

MAJOR FINDINGS

1. Significant differences between plasma HVA and plasma MHPG response to dexamethasone administration between depressed patients and controls was found. In comparison to metabolite levels on the pre-dexamethasone control day, normal controls showed a significant dexamethasone-induced increase in plasma levels of HVA and a trend towards a decrease in plasma levels of MHPG. Conversely, depressed patients, particularly those with psychotic features, showed a significant dexamethasone-induced increase in plasma MHPG and a decrease in plasma HVA relative to normal controls. Dexamethasone-induced increases in plasma MHPG were directly correlated with the severity of depressive symptoms and with post-dexamethasone cortisol levels in the depressed patients.
2. The administration of 80 mg of prednisone to normal volunteers resulted in subjective behavioral change in half of the subjects; behavioral effects included depressive symptoms in some and mild elation in other subjects. In addition to behavioral changes, prednisone produced a number of biological effects including significant reductions in CSF levels of somatostatin, beta-endorphin and beta-lipotropin.
3. In the studies of controllable and uncontrollable stress, despite the duration of noise exposure being identical for each subject on both test days, when exposed to uncontrollable stress subjects reported increased self-ratings of helplessness, lack of control, attention, stress, unhappiness, anxiety and depression as compared to either their baseline pre-stress ratings or following exposure to controllable stress. The behavioral changes observed following exposure to uncontrollable stress were also accompanied by increases in HPA axis function as measured by increases in plasma ACTH; the latter were highly correlated with increases in ratings of stress and tension and decrease in ratings of happiness. In pilot data from depressed subjects, uncontrollable stress appears to produce particularly pronounced increases in HPA axis function and in behavioral response in comparison to controllable stress. Analysis of catecholamine response (norepinephrine and epinephrine) is in progress.
4. The development of major depressive disorder in adulthood in patients who had experienced permanent early parental separation was high. The best predictors of adult depression was the quality of adjustment to the loss as determined by Homelife and Personal Adaptation (HAPA) Scale developed by the investigators. Plasma levels of cortisol and ACTH were correlated positively with HAPA scores, suggesting the possibility that early environmental trauma may be related to enduring neuroendocrine traits.
5. To date three patients with manic-depressive illness have completed the verapamil study. Verapamil appeared ineffective in either treating or preventing mania in the two patients who had bipolar I type illness. In the third patient, a rapidly cycling patient with bipolar II type illness, however, verapamil produced mood stabilization. Further studies are in progress.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

In this project we have focused on selected areas of the neurobiology of depression focusing on HPA function and stress. We have examined the CNS effects of steroids on behavior and on catecholamine metabolites and peptide transmitters. Results suggest abnormal corticosteroid-catecholamine interactions in depressed patients, particularly in those with psychotic features. These data are important as they contribute to the expanding body of data linking catecholamine and HPA axis abnormalities in depression. The prospective administration of oral prednisone to healthy volunteers has been a unique study which contributes both to the understanding of the behavioral effects of steroids, an important issue in medical practice, as well as steroidal interactions with neurobiologic systems. Preliminary analysis suggests that behavioral effects may be related to alterations of peptidergic systems.

The development of the controllable/uncontrollable stress paradigm is a unique contribution to research in the area of depressive illness. Our preliminary studies using this technique suggest that it may be an excellent tool for examining the role of stress on mood and catecholaminergic and HPA axis function in normal subjects. Its potential as a "model" of depression remains to be fully explored.

Our study suggests that the association between early parental separation and the development major depression in adulthood is critically dependent upon adjustment to loss. This project provides extremely important information which may help in adjusting children to parental loss. Moreover, findings suggest that adjustment to parental loss in childhood may play a role in determining activity of the HPA axis in adulthood.

The prospective evaluation of verapamil as a treatment for mania or hypomania will provide important information pertaining to the treatment of bipolar illness and provide excellent comparison for results already published from verapamil administration to schizophrenic patients.

PROPOSED COURSE

We plan to pursue the use of the controllable/uncontrollable stress paradigm to investigate the neurobiology of depression. We propose to investigate depressed and currently euthymic unipolar and bipolar patients. We have developed a modification of procedures to enhance the stress response. Pharmacologic intervention which might modify the response to uncontrollable stress are being evaluated.

PUBLICATIONS

Amsterdam, J.D., Henle, W., Winokur, A., Wolkowitz, O.M., Pickar, D., and Paul, S.M.: Serum antibodies to Epstein-Barr virus in patients with major depressive disorder. Am. J. Psychiatry 143(12):1593-1596, 1986.

Breier, A., Albus, M., Pickar, D., Zahn, T.P., Wolkowitz, O.M., and Paul, S.M.: Controllable and uncontrollable stress in humans: alterations in mood, neuroendocrine and psychophysiological function. Am. J. Psychiatry (in press).

- Breier, A., Kelsoe, J.R., Kirwin, P.D., Beller, S.A., Wolkowitz, O.M., and Pickar, D.: Early parental loss and the development of adult psychopathology. Arch. Gen. Psychiatry (in press).
- Roy, A. Five risk factors for depression. Br. J. Psychiatry 150:536-541, 1987.
- Roy, A., Agren, H., Pickar, D., Linnoila, M., Doran, A. R., Cutler, N. R., and Paul, S. M.: Reduced CSF concentrations of HVA and HVA to 5-HIAA ratios in depressed patients: relationship to suicidality and dexamethasone nonsuppression. Am. J. Psychiatry 143(12):1539-1545, 1986.
- Roy, A., Everett, D., Pickar, D., and Paul, S.M.: Platelet tritiated imipramine binding and serotonin uptake in depressed patients and controls: relationship to plasma cortisol levels before and after dexamethasone administration. Arch. Gen. Psychiatry 44:320-327, 1987.
- Roy, A., Guthrie, S., Pickar, D., and Linnoila, M.: Plasma norepinephrine responses to cold challenge in depressed patients and normal controls. Psychiatry Res. 21:161-168, 1987.
- Roy, A., Jimerson, D., Linnoila, M., Gold, P., and Pickar, D.: Premenopausal and postmenopausal depressed patients. Aust. NZ J. Psychiatry 20:464-469, 1986.
- Roy, A., Karoum, F., Linnoila, M., and Pickar, D.: Thyrotropin-releasing hormone test in unipolar depressed patients and controls: relationship to clinical and biologic variables. Acta Psychiatr. Scand. (in press).
- Roy, A., Linnoila, M., Karoum, F., and Pickar, D.: Relative activity of metabolic pathways for norepinephrine in endogenous depression. Acta Psychiatr. Scand. 73:624-628, 1986.
- Roy, A., Linnoila, M., Karoum, F., and Pickar, D.: Urinary excretion of free tyramine and norepinephrine and its metabolites in depression. Biol. Psychiatry 21:221-224, 1986.
- Roy, A., Pickar, D., Douillet, P., Karoum, F., and Linnoila, M.: Urinary monoamines and monoamine metabolites in subtypes of unipolar depressive disorder and normal controls. Psychol. Med. 16(3):541, 1986.
- Roy, A., Pickar, D., Linnoila, M., Chrousos, G.P., and Gold, P.W.: Cerebrospinal fluid corticotropin-releasing hormone in depression: relationship to noradrenergic function. Psychiatry Res. 20:229-237, 1987.
- Roy, A., Pickar, D., Paul, S., Doran, A., Chrousos, G.P., and Gold, P.W.: CSF corticotropin-releasing hormone in depressed patients and normal control subjects. Am. J. Psychiatry 144:641-645, 1987.
- Wolkowitz, O.M., Doran, A.R., Breier, A., Roy, A., Jimerson, D.C., Sutton, M.E., Golden, R.N., Paul, S.M., and Pickar, D.: The effects of dexamethasone on plasma homovanillic acid and 3-methoxy-4-hydroxyphenylglycol: evidence for abnormal corticosteroid-catecholamine interactions in major depression. Arch. Gen. Psychiatry 44:782-789, 1987.

Wolkowitz, O.M., Rubinow, D.R., Breier, A., Doran, A.R., Davis, C., and Pickar, D.: Prednisone decreases CSF somatostatin in healthy humans: implications for neuropsychiatric illness. Life Sciences (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

701 MH 02187-04 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Diazepam Infusions as a Measure of Benzodiazepine Receptor Sensitivity in Humans
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D.W. Hommer	Staff Psychiatrist	NS, NIMH
Others:	S.M. Paul	Chief	NS, NIMH
	D. Pickar	Chief, Section on Clinical Studies	NS, NIMH
	A. Breier	Medical Staff Fellow	NS, NIMH
	H. Weingartner	Chief, Section on Psychopathology	LPP, NIMH

COOPERATING UNITS (if any)

Laboratory of Psychology and Psychopathology, NIMH; Alcohol Intramural Research Program, National Institute of Alcohol Abuse and Alcoholism; Tufts University

LAB/BRANCH
Clinical Neuroscience BranchSECTION
Section on Clinical StudiesINSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	1.5	PROFESSIONAL:	1.0	OTHER:	0.5
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CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Increasing doses of the benzodiazepine, diazepam, or placebo were administered to normal volunteers, drug-free alcoholic inpatients. In one series of experiments, subjects were pretreated with either placebo or the benzodiazepine antagonist, Ro-15-1788. Following each dose of drug saccadic eye velocity, diazepam blood levels, plasma cortisol, and growth hormone were measured and self-ratings of anxiety and sedation were performed. After every other dose cognitive testing of memory and attention was performed. The effects of diazepam on these variables was quantified and diazepam dose response curves constructed. These dose response curves provide a measure of benzodiazepine receptor sensitivity in humans, as well as an evaluation of the ability of specific antagonists to block the actions of benzodiazepines.

OTHER PROFESSIONAL PERSONNEL

M. Linnoila Clinical Director
D. Greenblatt Tufts University School of Medicine

ALC, NIAAA

PROJECT DESCRIPTION

We have been examining the ability of two BZ antagonists, the specific antagonist Ro-15-1788 and the non-specific antagonist caffeine, to block the effects of intravenous diazepam. Ro-15-1788 is given i.v., 0.035 mg/kg. Caffeine is given orally either 3 or 10 mg/kg. Then diazepam is administered intravenously in doses of 25, 25, 50, and 100 µg/kg at 15 minute intervals. After each dose measurement of a variety biological and psychological variables that are affected by benzodiazepines is made. These variables include: memory and attention (done with Dr. Herbert Weingartner), velocity of saccadic eye movements growth hormone, cortisol, and self-ratings of anxiety and sedation.

MAJOR FINDINGS

Plasma cortisol, saccadic eye velocity, self-rated sedation and recent memory all significantly decrease during diazepam administration while growth hormone concentration is increased significantly in most, but not all, individuals. From this data we constructed diazepam dose-response curves. These dose-response curves provide an in vivo measure of BZ receptor sensitivity in humans. Furthermore, we found extremely high correlations between all these variables during diazepam administration suggesting that these disparate effects are all mediated through the same class of BZ receptors as well as a high correlation between changes in these variables and increasing plasma concentrations of diazepam. Ro-15-1788 also blocks sedation, anxiety and decreases in saccadic eye velocity. However, it failed to block diazepam-induced decrease cortisol or recent memory. In contrast Ro-15-1788 did block BZ induced changes in growth hormone and attention.

In addition to studying BZ receptor sensitivity in normals we have also examined BZ receptor sensitivity in 10 chronic alcoholic subjects who had been alcohol and drug-free for one month at the time of the study. These subjects showed a slightly greater sensitivity to diazepam than the normal controls.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

Benzodiazepines are the most commonly prescribed psychotropic drugs in the world. The recent identification of stereospecific receptors for BZs in the brain of both animals and humans raises several new and exciting avenues of research not only into the mechanism of action of these anxiolytic and anticonvulsant agents but also into the pathophysiology of anxiety itself. For example, studies in rats have demonstrated that stress changes brain BZ receptors. If a similar phenomena occurs in humans it may provide the etiological link between stress and mental illnesses such as the anxiety, depression and post-traumatic stress disorder. In order to study BZ receptor sensitivity in psychiatric illness it is first necessary to develop a valid and reliable measure of BZ receptor sensitivity in normal human subjects. This project has provided such a measure of BZ receptor sensitivity and our preliminary results suggest that BZ receptor sensitivity may be altered in alcoholism.

PROPOSED COURSE

No further studies are planned since principal investigator is leaving NIMH.

PUBLICATIONS

Hommer, D. W., Matsuo, V., Wolkowitz, O. M., Weingartner, H., and Paul, S. M.: Pharmacodynamic approaches to benzodiazepine actions in man. Proceedings of the 4th International Meeting on Clinical Pharmacology in Psychiatry, Selectivity in Psychotropic Drug Action--Promises or Problems, Vol. 3; Srpinger-Verlag, 1986, pp. 52-61. Bethesda, MD (in press).

Weingartner, H., Thompson, K., Wolkowitz, O., and Hommer, D.: Neuropharmacological analysis of distinct types of memory processes. Psychopharmacology (in press).

Wolkowitz, O. M., Weingartner, H., Thompson, K., Pickar, D., Paul, S. M., and Hommer, D. W.: Diazepam-induced amnesia a neuropharmacological model of an "organic amnestic syndrome." Am. J. Psychiatry 144:25-29, 1987.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02188-03 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological Studies of Borderline Personality Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D.L. Gardner	Staff Psychiatrist	OCD, NIMH
Others: R.W. Cowdry	Clinical Director	NIMH
K.M. O'Leary	Social Worker (Research)	OCD, DIRP, NIMH
D. Pickar	Chief, Sec. on Clinical Studies	NS, NIMH
P. Lucas	Clinical Fellow, NRSA Fellow	NS, NIMH
D.L. Murphy	Chief	LCS, NIMH

COOPERATING UNITS (if any)

Office of the Director, Division of Intramural Research Program, NIMH;
Biological Psychiatry Branch, NIMH; Laboratory of Clinical Science, NIMH

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

1.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been transferred to Office of the Clinical Director (OCD).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00179-06 NS
PERIOD COVERED October 1, 1985 to September 30, 1986		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Morphological Aspects of Peptides in Mammalian Brain		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: L. Skirboll Sr. Staff Fellow NS, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Unit on Electrophysiology, Section on Molecular Pharmacology		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) PROJECT HAS BEEN DISCONTINUED DUE TO PRINCIPAL INVESTIGATOR'S REASSIGNMENT.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02177-05 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Functions of Neuropeptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.N. Crawley	Senior Staff Fellow	NS, NIMH
Others:	L.R. Skirboll	Senior Staff Fellow	NS, NIMH
	D.W. Hommer	Staff Psychiatrist	NS, NIMH
	J. Mastropalo	Staff Fellow	NS, NIMH
	S.M. Paul	Chief	NS, NIMH
	D.M. Jacobowitz	Section Chief	LCS NIMH

COOPERATING UNITS (if any) Unit on Electrophysiology, Section on Molecular Pharmacology, NS and Section on Histopharmacology, Laboratory of Clinical Science, NIMH

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The past decade has witnessed the discovery of forty or more peptides localized in neurons of mammalian brain. Many cases of peptides coexisting in the same neuron with classical transmitters have been described. Our laboratory is investigating the functional significance of coexisting peptides and transmitters in the central nervous system, using behavioral tools.

A) We previously showed that cholecystokinin (CCK) potentiates dopamine-induced hyperlocomotion in the nucleus accumbens, the terminal region of the mesolimbic pathway where CCK and DA coexist. This year, we began to characterize the role of CCK on DA autoreceptors in the ventral tegmentum (VTA), the cell body region of the mesolimbic pathway where CCK and DA coexist. Preliminary data show that CCK potentiates the hypolocomotion induced by DA in the VTA, while having no effect alone, i.e. the potentiating effect of CCK in the VTA was not blocked by doses of proglumide which blocked the potentiating effect of CCK in the nucleus accumbens. In addition, the unsulfated form of CCK-8 was also active in potentiating DA-induced hypolocomotion in the VTA. These preliminary behavioral data suggest that the pharmacology of CCK effects in the VTA match the "central-type" CCK-receptor, as found by Skirboll and Hommer using electrophysiological techniques. Conversely, the pharmacology of CCK effects in the nucleus accumbens match the "peripheral-type" CCK receptor in our previous behavioral data.

B) The nucleus basalis of Meynert lesion rat model of Alzheimer's disease is now ongoing in our laboratory. We have completed one experiment showing that acetylcholine (ACH) can reverse the memory deficit in a T-maze task when given directly into the lateral ventricles, the first demonstration of a central site of action for ACH. The second experiment found that atropine, but not mecamylamine, blocked the ACH effect, suggesting the involvement of a muscarinic, rather than a nicotinic cholinergic receptor. Tests of somatostatin and galanin, alone and in combination with a submaximal dose of ACH, are in progress.

OBJECTIVES

Behavioral analysis of the functions of neuropeptides, particularly the functional significance of peptides coexisting with transmitters in mammalian brain.

METHODS

(SEE: 1985 Annual Report, Project Number Z01 MH 02177-03 NS, pp 503-507)

Aseptic stereotaxic implantation of indwelling cannulae into brain nuclei of rats. Microinjection of neurochemicals into discrete anatomical nuclei. Intra-peritoneal injection of drugs. Behavioral analysis of neurochemical effects, including locomotion, seizures, grooming, stereotyped motor behavior, and T-maze alternation task. Histological verification of cannulae placement and injection sites.

MAJOR FINDINGS

This year we initiated studies on the actions of cholecystokinin, (CCK) in the ventral tegmental area (VTA) on the cell bodies of mesolimbic neurons within which CCK and dopamine (DA) coexist. CCK microinjected into the VTA, had no effect alone on locomotion, at doses from 100pg-100ng. CCK significantly potentiated the hypolocomotion into the VTA, at doses of 1ng-400ng CCK. Unsulfated CCK, 100ng, also potentiated DA-induced hypolocomotion in the VTA. Proglumide, 10mg/kg, did not block the ability of CCK, 100ng, to potentiate DA-induced hypolocomotion. The potency of unsulfated CCK-8, and the lack of action of proglumide, are consistent with the binding characteristics of the "central-type" CCK receptors. These behavioral actions of CCK are consistent with the electrophysiological data of Lana Skirboll and Dan Hommer, from extracellular recording in the VTA. However, the behavioral profile of CCK in the VTA differs from the behavioral profile of CCK in the nucleus accumbens, where unsulfated CCK-8 is inactive, and proglumide is an effective antagonist. These preliminary data suggest that CCK modulates DA in a facilitatory manner, at a "peripheral-type" receptor at the post-synaptic site in the nucleus accumbens, and at a "central-type" receptor at the cell body autoreceptor site in the ventral tegmentum.

The ability of oxytocin to stimulate grooming behaviors when microinjected into the ventral tegmental area was characterized in terms of dose, time course, influence of sex hormones, antagonism by an oxytocin antagonist, and antagonism by dopamine receptor antagonists. In addition, doses of oxytocin which induced grooming, considered a stress-related behavior, were found to have no effect on locomotion. Most peptides affect locomotor activity when administered into the VTA. Oxytocin may be a useful tool for delineating subpopulations of neurons within the VTA with projections to brain regions other than the nucleus accumbens, e.g. the prefrontal cortex.

Several neuropeptides have been reported to decline in the brains of patient with advanced Alzheimer's disease. The largest changes are in cerebral cortex regions innervated by the nucleus basalis of Meynert, which degenerates in Alzheimer's disease. To investigate the possible role of neuropeptides as potential treatments for Alzheimer's disease, we are in the process of setting up the nucleus basalis of Meynert lesion model of Alzheimer's disease in rats. Dr. John Mastropaolo joined our laboratory on July 7, 1986, to develop this project. A standard t-maze alternation task was designed, built, and implemented. Dr. Mastropaolo determined the best stereotaxic coordinates, the correct dose of libotenic acid, the necessary number of lesion sites, and the minimum intertrial delay, to optimize the difference in performance between lesioned and sham control rats in the t-maze task. His first experiment showed that intraventricular acetylcholine (ACH) could reverse the memory deficit created by the nbM lesion. dose-response curve for ACH showed a maximal effect of 10 μ g, with doses of 20 μ g and above inducing seizures as we had previously reported for carbachol. The restorative effects of ACH were blocked by atropine but not mecamylamine, demonstrating that the ACH was acting through a muscarinic, rather than a nicotinic, cholinergic receptor. These data are important for two reasons: 1) they provide the first direct demonstration that ACH can reverse memory deficits at a central site (all previous studies used systemic injections of cholinergic drugs); 2) they provide the first direct demonstration of the importance of the muscarinic receptor in memory processes after nbM lesions (most previous studies of central cholinergic neurons in animal memory tasks implicated nicotinic receptors). Current experiments are testing somatostatin and galanin, alone and in combination with a submaximal dose of ACH, in this paradigm.

PROPOSED COURSE

All studies on peptide modulation of transmitter function will be continued, to address questions of anatomical and neurochemical specificity, role of the endogenous peptides, and testing of non-peptide analogs and antagonists as putative new therapeutic drugs.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

The basic research questions being answered center around the activity of neuropeptides as either primary neurotransmitters or modulators of primary neurotransmitters. The clinical applications of our results may be far-reaching. If endogenous CCK is found to modulate endogenous dopamine in the mesolimbic pathway, then new antipsychotic drugs could be developed from non-peptide antagonists of CCK. Several drug companies, including Merck, Abbott, Ciba-Geigy and Rotta are presently developing such antagonists. A combination of lower dose of a DA antagonist, with a CCK antagonist, could reduce the symptoms of schizophrenia without as great a risk for tardive dyskinesias. If peptides reverse the memory deficits induced lesions of the nucleus basalis of Meynert, then new treatments for Alzheimer's disease could be developed from non-peptide analogs or antagonists.

Blumstein, L. K., Crawley, J. N., Davis, L. G., and Baldino, F.: Neuropeptide modulation of apomorphine-induced stereotyped behavior. Brain Res. 404:293-300 1987.

Crawley, J. N., Stivers, J. A., and Jacobowitz, D. M.: Neuropeptides modulate carbachol-stimulated "boxing" behavior in the rat medial frontal cortex. In Moody, T. W. (Ed.): Neural and Endocrine Peptides and Receptors, Plenum Press, NY 1986.

Crawley, J. N.: Modulation of mesolimbic dopaminergic behaviors by cholecystokinin. The Mesocorticolimbic Dopamine System. Annals of the York Academy of Sciences, in press.

Crawley, J. N.: Behavioral analysis of the functional significance of peptide-transmitter coexistences. Receptor-Receptor Interactions, ed. K. Fuxe and L. Agnati, in press.

Crawley, J. N., Hommer, D.W. and Skirboll, L.R.: Cholecystokinin-dopamine interactions: electrophysiological and behavioral studies. Sixth International Catecholamine Symposium, Alan R. Liss, Inc. NY, in press.

Hommer, D. W., Stoner, G., Crawley, J. N., Paul, S. M. and Skirboll, L. R.: Cholecystokinin-dopamine coexistence: electrophysiological actions corresponding to cholecystokinin receptor subtype. J. Neurosci. 6:10:3039-3043, 1986.

Kaltwasser, M. T., Petrack, B. and Crawley, J. N.: Potency of CR 1409, a new proglumide analog, on cholecystokinin-mediated behaviors and receptor binding. Neurochemistry International, 10:4:547-553, 1987.

Kaltwasser, M. T. and Crawley, J. N.: Oxytocin and cholecystokinin induce grooming behavior in the ventral tegmentum of the rat. Brain Research, (in press).

Skirboll, L. R., Crawley, J. N., and Hommer, D. W.: Functional studies of CCK-coexistence electrophysiology and behavior. In Hokfelt, T., Pernow, B., and Fuxe K. (Eds.): Coexistence, Progress in Brain Research, volume 68, 1986.

Stivers, J. A. and Crawley, J. N.: Substance P antagonists block carbachol-induced "boxing" behavior at a site of coexistence in the rat prefrontal cortex Peptides, in press.

Stivers, J. A., Kaltwasser, M. T., Hill, P. S., Hruby, V. J. and Crawley, J. N. Ventral tegmental oxytocin induces grooming. Peptides, in press.

Stivers, J. A., Skirboll, L. R., Long, R. and Crawley, J. N.: Anatomical analysis of frontal cortex sites at which carbachol induces motor seizures in the rat. Peptides, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02178-04 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology of Anxiety

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.N. Crawley	Senior Staff Fellow	NS, NIMH
	R.C. Drugan	NRSA Fellow	NS, NIHM
Others:	P. Skolnick	Pharmacologist	LBC, NIADDK
	Leslie Morrow		NS, NIMH
	Steve Deutsch		NS, NIMH
	Avi Weizman		NS, NIMH
	Ronit Weizman		NS, NIMH
	Steven M. Paul	Chief	NS, NIMH

COOPERATING UNITS (if any)

Unit on Electrophysiology, Section on Molecular Pharmacology, NS, NIMH;
Laboratory on Bioorganic Chemistry, NIADDK.

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Animal models of anxiety are being used to investigate the biological mechanisms underlying anxiety-related behaviors. The anxiety component of the learned helplessness model of depression was characterized using in vivo binding of ³H-Ro15-1788 and in assays of chloride flux in brain regions from rats experiencing escapable versus inescapable stressors.

OBJECTIVES

Characterization of changes in neurotransmitters, receptors and effector system induced by discrete behavioral events which may serve as models for human anxiety states.

METHODS

(SEE: 1985 Annual Report, Project Number Z01 MH 02178-03 NS, pp 509-511)

Mouse exploration test, rat learned helplessness test, receptor binding assays for radiolabeled benzodiazepines, GABAergic drugs, and chloride channel ligands Assays of radiolabeled chloride flux.

MAJOR FINDINGS

Dr Rob Drugan, a postdoctoral fellow in our Unit, has continued to analyze neurochemical correlates of the learned helplessness paradigm. He has found a large decrease in ^{35}S -TBPS binding in cerebral cortex and hippocampus of rats who experienced inescapable tailshock but did not develop the syndrome, suggesting that "coping" behavior requires changes in GABAergic function which do not occur in "non-copers". These data also suggest that the chloride channel is the most sensitive indicator for the benzodiazepine-GABA-chloride ionophore receptor complex, since no changes in ^3H -flunitrazepam binding, and only minor changes in ^3H -muscimol binding, were seen in rats who showed large decreases in ^{35}S -TBPS binding. To further investigate the comparative indices of the chloride channel versus the benzodiazepine binding site, two more powerful techniques are presently being employed. In collaboration with Leslie Morrow, Steven Paul's lab, Rob is finding changes in chloride-18 flux in cerebral cortex from rats developing the learned helplessness syndrome. In collaboration with Steve Deutsch, Ronit Weizman and Avi Weizman, in Steven Paul's lab, Rob is finding significant changes in ^3H -Ro-1788 binding when this radioligand for the brain benzodiazepine receptor is injected intravenously, after the learned helplessness procedure and before sacrifice.

In addition, in collaboration with Phil Skolnick, Rob is investigating the mechanism by which inescapable tailshock reduces binding of ^3H -Ro15-1788 to rat kidney. Neither hypophysectomy nor adrenalectomy blocked this effect, suggesting that behavioral stressors affect this peripheral-type benzodiazepine receptor by a mechanism other than the hypothalamic-pituitary-adrenal-axis.

PROPOSED COURSE

Rob will be completing the ongoing studies with chloride flux, in vivo ^3H -Ro-17887 binding and ^3H -Ro-4864 binding in the learned helplessness paradigm. A new post-doctoral PRAT fellow will join our Unit in August, to continue this program. Sandra Cottingham will use all of our behavioral and biochemical assays to characterize the Maudsley Reactive rat, bred for "hyperemotionality". We hope that she will also test this substrain for genetic differences in structure or regulation of the genes for tyrosine hydroxylase and gamma aminobutyric acid, in collaboration with Ed Ginns in our branch. Maudsley Reactive and Non-reactive rats are currently breeding in our branch vivarium for these studies.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

Our work on neural mechanisms mediating anxiety is designed to identify brain transmitters, receptors, effector systems, and genetic factors which may be responsible for syndromes of extreme and chronic anxiety.

PUBLICATIONS

Crawley, J. N., Glowa, J. R., Majewska, M. D., and Paul, S. M.: Anxiolytic activity of an endogenous steroid. Brain Res. 398:382-385, 1986.

Drugan, R. C., Basile, A. S., Crawley, J. N., Paul, S. M., and Skolnick, P.: Inescapable shock reduces [³H] Ro5-4864 binding to "peripheral-type" benzodiazepine receptors in the rat. Pharmacol. Biochem. Behav. 24:1673-1677, 1986.

Drugan, R. C., Basile, A. S., Crawley, J. N., Paul, S. M., and Skolnick, P.: "Peripheral" benzodiazepine binding sites in the Maudsley Reactive Rat: Selective decrease confined to peripheral tissue. Brain Research Bulletin 18:143-145, 1987.

Paul, S. M., Crawley, J. N., and Skolnick, P.: The neurobiology of anxiety. American Handbook of Psychiatry (in press).

Drugan, R. C., Crawley, J. N., Paul, S. M. and Skolnick, P: Buspirone attenuate learned helplessness behavior in rats. Drug Devel Res. 10:63-67, 1987.

Smith, C. B. and Crawley, J. N.: Anxiolytic action of CGS 9896 on mouse exploratory behavior. Europ. J. Pharmacol. 132:259-262, 1986.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02179-05 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Animal Models of Neuropsychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J.N. Crowley	Senior Staff Fellow	NS, NIMH
Others:	S.M. Paul	Chief	NS, NIMH
	P. Suzdak	Staff Fellow	NS, NIMH
	G. Wand	Asst. Professor	
		Dept of Neuroscience, Johns Hopkins	
		University School of Medicine	

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Characterization of a benzodiazepine, Ro15-4513, which antagonizes the biochemical actions of ethanol in the rat, was performed on the behavioral test for intoxication. Other inverse agonists such as FG-7142 and β -CCE did not block the intoxicating effects of ethanol, suggesting that Ro15-4513 acts through a mechanism which is independent of any direct behavioral effects of inverse agonists of the central benzodiazepine receptor.

Preliminary assays of neuropeptides in the hypothalamus of dwarf hamsters found increased levels of NPY in separated males. Microinjection of NPY in rats is known to stimulate feeding; separated male dwarf hamsters have increased body weight.

OBJECTIVES

Development and evaluation of a new hamster model of depression. Application of animal models to study the biochemical basis of neuropsychiatric disorders including depression, schizophrenia, obesity and alcoholism.

METHODS

(SEE: 1985 Annual Report, Project Number Z01 MH 02179-03 NS, pp 513-515)

Breeding, pairing, separating, and neurochemical assays of Siberian dwarf hamsters. Behavioral testing for locomotion, feeding, and alcohol-induced intoxication. Chronic drug treatments, cannulation and microinjection into discrete anatomical loci.

MAJOR FINDINGS

Ro15-4513, was found to both prevent and reverse the intoxication produced by 2g/kg ethyl alcohol in a dose-related fashion. Lower doses, of Ro15-4513 were required to prevent than to reverse intoxication. Intoxication induced by methanol and by t-butyl alcohol was also prevented Ro15-4513. The benzodiazepine receptor antagonists, Ro15-1788 and CGS 8162, blocked the actions of Ro15-4513 on both prevention and reversal of alcohol intoxication. Two other inverse antagonists of the central benzodiazepine receptor failed to reverse ethanol-induced intoxication, FG-7142 (10 mg/kg and 30 mg/kg) and β -CCE (10 mg/kg). However, combined treatment of Ro4513 and β -CCE produced competitive interactions. High doses of Ro15-4513 failed to reverse intoxication induced by high doses of ethanol (4 grams/kg). These data suggest that Ro15-4513 can effectively inhibit alcohol-induced intoxication at an unknown site which is different than, but linked to, the site at which Ro15-4513 and other inverse agonists bind to the central benzodiazepine receptor.

In collaboration with Dr. Gary Wand, several peptides have been assayed in pituitary hypothalamus and cortex of paired and separate male dwarf hamsters. Separated males were found to have significantly lower levels of ACTH in the pituitary, and significantly higher levels of neuropeptide Y in the hypothalamus. These preliminary data are interesting in that separated males are less active and have higher body weights than paired males. Neuropeptide Y has been found to be the most potent peptide which increases feeding behavior in male rats.

PROPOSED COURSE

The behavioral analysis of Ro15-4513 is being completed for publication. Further testing of this class of drugs for antagonism of alcohol-induced intoxication will be undertaken if such compounds become available.

Replications of assays for NPY, ACTH, and CRF are in progress in paired and separated dwarf hamsters, to determine the importance of these neuropeptides for the "separation syndrome".

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

Our Unit is the only laboratory in NIMH for the development of new animal model of neuropsychiatric disorders. We continue to investigate the biological bases of dysfunctions including depression, schizophrenia, anxiety, obesity, and alcoholism. Our applications goal is to help develop rational, specific drugs for these illnesses.

PUBLICATIONS

Angel, I., Kiss, A., Stivers, J. A., Skirboll, L. R., Crawley, J. N., and Paul, S. M.: Regulation of [³H]mazindol binding to subhypothalamic areas: involvement in glucoprivic feeding. Brain Res. Bull. 17:873-877, 1986.

Angel, I., Stivers, J. A., Paul, S. M. and Crawley, J. N.: Site of action of anorectic drugs: glucoprivic- versus food deprivation-induced feeding. Pharmacol. Biochem. Behav., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02180-05 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Electrophysiological Studies of Peptidergic and GABAergic Function in Mammalian Brain.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. Hommer	Staff Psychiatrist	NS, NIMH
Others:	L. Skirboll	Sr. Staff Fellow	NS, NIMH
	J.N. Crawley	Sr. Staff Fellow	NS, NIMH
	S.M. Paul	Chief	NS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Unit on Electrophysiology, Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Using extracellular single unit recording techniques, we have examined the effects of stress and pharmacological agents which either alleviate or mimic the effects of stress on individual neurons in the rat substantia nigra (SN). Specifically, learned helplessness induced by uncontrollable stressful shocks results in a supersensitivity to gamma-amino butyric acid (GABA) agonists while shocks which are controllable do not produce GABAergic supersensitivity. The anxiogenic benzodiazepine (BZ) receptor ligand, beta-carboline carboxylate ethyl ester (βCCE) increases the activity of neurons in the SN zona reticulata (ZR) but had no effect on noradrenergic neurons in the locus coeruleus. Caffeine also mimics many of the effects of βCCE in the SN but its actions are not reversed by the specific BZ antagonist Ro15-1788 as are those of βCCE. Furthermore, the effects of caffeine could be blocked by lesions of the caudate nucleus while the effects of βCCE were unaffected by such lesions.

We have continued our studies on the interactions between endogenously occurring neuropeptides and classical neurotransmitters. All varieties of CCK-like peptides which bind to brain CCK receptors also potentiate DA in those areas where CCK and DA coexist while those CCK-like peptides which do not bind to this receptor are ineffectual in facilitating DA inhibition. The putative cholecystokinin (CCK) antagonists, proglumide and benzotript, were found to weakly block CCK in proportion to their potency at central CCK receptors.

Dynorphin (DYN) appears to modulate the response of SNZR neurons to GABA. Finally, we have examined the effects to cholinergic agents in the SN. Nicotinic agents appear to activate DA neurons in the SN through a central mechanism.

PROJECT DESCRIPTIONOBJECTIVES

The SNZR is a region of the brain which contains a high concentration of GABA, BZ receptors as well the opiate-like peptide, DYN. Recently several groups have shown that microinjections of GABA agonists into the SN, but not into adjacent mesencephalic areas, blocks electrically-induced seizures in rats. These microinjections also block the limbic afterdischarge following kindled seizures. This suggests that the SN may be an important region involved in the propagation of seizure activity as well as in modulation of limbic system function. Furthermore, since GABA, BZ's and opiates all have been implicated in the neural response to stress, the SN represents an excellent system in which to study the effects of stress as well as the drugs and putative transmitters which may modulate stress and anxiety.

Aside from peptides and GABA present in the SN, it has also been reported that the SN is an area in nicotinic receptors. In this regard, we have been examining the cholinergic influence on the activity of nigral neurons. We have explored the effects of nicotine on the firing of neurons in the SNZR and SN zona compacta (ZC) neurons.

Immunohistochemical studies have shown that there is a coexistence of DA and CCK in a subpopulation of mesencephalic neurons in the rat which project primarily to limbic areas. We have previously reported that these DA-CCK containing cells are excited by either systemically or iontophoretically administered CCK, we have investigated the question of the functional significance of this coexistence (i.e. how does the peptide CCK and the classical neurotransmitter, DA, interact to affect the activity of neurons). We have also investigated the interaction between GABA and peptide dynorphine (DYN) in the SNZR. These experiments provide another avenue toward developing an understanding of the functional relationship between neurotransmitter systems.

METHODS

(SEE: 1985 Annual Report, Project Number Z01 MH 02180-03 NS, pp 517-521)

MAJOR FINDINGS

1. Effects of stress on GABAergic sensitivity in the SNZR.

When rats are subjected to tail shock which they cannot control, this experience produces a supersensitive response to the intravenously administered GABA agonist, muscimol. This effect appears to be related to the uncontrollable nature of the shock. Animals which are subjected to an identical shock exposure but over which they have some control do not develop a supersensitive response to muscimol. Thus, uncontrollable stress leads to an increased sensitivity of SNZR neurons to the inhibitory actions of GABAergic agents. This GABAergic supersensitivity is present immediately after termination of the uncontrollable shock and persists for at least 2 hours. It is not prevented by adrenalectomy.

2. Effects of drugs which may mimic the effects of stress.

Caffeine decreases the activity of DA neurons in the ventral tegmental (VT) area which project to limbic regions but has little effect on DA neurons in the more lateral SN regions which project to the striatum. This caffeine-induced inhibition can be blocked by either diazepam or haloperidol suggesting that both the BZ receptor and DA release may play a role in mediating caffeine's actions. Caffeine also produces an excitatory effect in the SNZR. This excitation can be blocked by lesions of the caudate nucleus suggesting that the pro-convulsant anxiogenic actions of caffeine are at least in part mediated through the basa ganglia. In a separate series of experiments we have examined the effects of a intravenously administered alprazolam, a high-potency triazolo-benzodiazepine, on the activity of A9 and A10 DA neuron. Alprazolam had a small dose-dependent excitatory effect on the DA neurons which project to the striatum in contrast it demonstrated a marked, dose-dependent, excitatory effect on DA neurons in A10. It appears that the cell bodies of the mesolimbic and mesocortical DA systems are much more sensitive to BZ effects than is the nigrostriatal system.

3. CCK-DA interactions

As we have previously reported, ceruletide and sulfated CCK-8 (CCK-8-US) both potentiate the effects of the DA agonist, apomorphine, on DA neurons in the medial SN. We have also found that unsulfated CCK-8 (CCK-8-US) and CCK-4 posse a similar ability to potentiate apomorphine induced inhibition in the SN. The rank order potencies were as follows: ceruletide > CCK-8-S = CCK-8-US > CCK-4; CCK-3 was without effect. These potencies directly parallel the affinity of these peptides for the brain CCK receptor. In contrast, only CCK-8-S produced excitatory effect on DA neurons while CCK-US, CCK-4 and CCK-3 were devoid of effect. This profile of activity corresponds to the affinities of the various CCK-like peptides at the peripheral CCK binding site. It appears that CCK's different CNS effects may be mediated by different CCK receptors.

4. GABA-DYN interactions.

We have found that DYN, an opiate-like peptide present in high concentrations the SNZR modulates the effects of the neuronal inhibition produced by GABA applied directly to nigral neurons. DYN had two distinct actions; either potentiation or attenuation of the effects of GABA. In cells which were themselves inhibited by DYN, the peptide potentiated GABA's actions. In those cells which were unresponsive to DYN, simultaneous application of DYN and GABA resulted in an attenuation of the GABA inhibition. These findings provide further evidence for the modulatory interaction between peptides and classical neurotransmitters. None of the effects of DYN could be blocked by mu or sigma opiate receptor antagonists.

5. Nicotinic influences on nigral activity.

Low systemic doses of nicotine stimulated the firing of SNZC DA cells; experiments with antagonists showed this action to be due to a central and probably direct action of nicotine on these cells. Nicotine also excited non-DYN cells in the SNZR, but this action was clearly of peripheral origin.

These results are in accord with autoradiographic data showing that nicotinic binding sites in the SN are largely restricted to the SNZC.

PROJECTED COURSE

We plan to continue our investigations of the effects of stress in the SN and expand our efforts to include examinations of other neurotransmitters such as DYN and enkephalins as well as GABA. We also plan to more fully characterize the phenomenon of increased sensitivity to GABAergic agents through the use of iontophoretic techniques and an examination of the role of stress related hormones such as the corticosteroids.

Studies of the interactions between classical transmitters and peptides will be extended along several lines. First, the modulation of the GABA response by DYN will be compared to other peptide like substance P and the enkephalins. Secondly, the hypothalamus will be explored as a site of multiple transmitter interaction. In immunohistochemical studies (see Z01 MH 00179-04 NS), we have been exploring the transmitter systems which innervate the paraventricular nucleus. These transmitters can be altered by adrenalectomy or systemic administration of a phenyl-n-methyl-transferase inhibitor. We plan to explore the functional correlate of these findings. That is, look at the electrophysiologic response of paraventricular nucleus neurons to neuropeptide adrenaline and adrenalectomy.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

Studies of the interaction between transmitters (CCK-DA and GABA-DYN) may provide a prototype for modulatory function between peptide and classical transmitters. Furthermore, electrophysiological studies of BZ and GABA complement ongoing biochemical and behavioral studies in these areas. The SN appears to be an important brain region in the modulation of seizures and some aspects of anxiety. Electrophysiological techniques are particularly well suited to further our understanding of how the SN performs this function. In addition, the hypothalamus is the site of control of many vegetative and neuroendocrine functions which control response to stress. Thus, understanding how stress affects the nigral and hypothalamic systems may be of potential value in developing pharmacological approaches to stress related diseases, both medical and psychiatric.

PUBLICATIONS

Clarke, P., Hommer, D., and Skirboll, L.: Stimulation of cholinergic projection to substantia nigra increase neuronal activity. Brain Res. (in press).

Clarke, P. B. S., Pert, A., Hommer, D. W., and Skirboll, L. R.: Electrophysiological actions of nicotine on substantia nigra single units. Br. J. Pharmacol. 85:827-835, 1985.

Hommer, D., Stoner, G., and Skirboll, L.: CCK-DA coexistence: electrophysiological actions corresponding to CCK-receptor subtype. J. Neurosci. 6:3039-3043, 1986.

Skirboll, L. R., Crawley, J., and Hommer, D.: Functional studies of CCK-DA coexistence: electrophysiology and behavior. In Hokfelt, T., Pernow, B., and Fuxe, K. (eds). Progress in Brain Research, 681, (in press).

Stoner, G., Skirboll, L., and Hommer, D.: Differential effects of an anxiogenic β -carboline on single unit activity in the locus coeruleus and substantia nigra of rat brain. Neuropharmacology, (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02182-05 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Toward the Visualization of Opiate Receptors in Living Humans

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. B. Pert Guest Researcher NS, NIMH

Others: N. L. Ostrowski Senior Staff Fellow LCM, NIMH
K. C. Rice Pharmacologist LC, NIDDK

COOPERATING UNITS (if any)

Laboratory of Chemistry, NIDDK

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

2.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The distribution of positron emitting substances in brain can be followed by positron emission tomography (PET). We are developing ^{18}F -labeled high affinity opiate drugs to be injected into living humans for the visualization of opiate receptor patterns in vivo. It will be interesting to determine whether opiate receptor distribution patterns in cortex change as a function of attention and emotional states. Meanwhile, we have carefully documented the receptor-binding properties and kinetics of ^3H -cyclofoxy in rat brain after in vivo injection.

Project Description:Objectives:

To demonstrate gradients of opiate receptor density in the cortex of living humans. To examine whether differences in these gradients exist as a function of emotional state or attentional processes.

Methods Employed:

PET Scan--using newly developed ^{18}F -labeled opiate analogs. Autoradiography of rat brain slices.

Major Findings:

We managed to affix a fluoride moiety to naltrexone, a potent opiate antagonist without losing affinity for opiate receptors. This fluoro-opiate derivative is suitable for in vivo injections for visualizing receptors. We have visualized stereospecific, striking images for opiate receptors in the thalamus, basal ganglia and frontal cortex of a living baboon. Tritium labeled cyclofoxy has been prepared and shown in rats to have the appropriate opiate distribution pattern.

Significance to Biomedical Research and Program of the Institute:

The notion that alterations in mood are a function of oscillations in neurotransmitter receptor sensitivity is perhaps the most exciting new lead in attempting to understand the causes of mental illness. Other leads in this institute point to the relevance of cortical participation as a critical factor in psychiatric disease. The opiate receptor is the most well-studied of brain receptors and appears to be associated with the pleasure of fulfilled appetite.

Proposed Course:

Our new useful probe, $[\text{}^3\text{H}]\text{-3-acetylcyclofoxy}$, will be characterized thoroughly in rodents to fulfill requirements for human use. Human studies, first on normal controls, then psychiatric patients, should enable a rigorous test of theories of emotions which emphasize neuropeptide receptors.

Publications:

1. Ostrowski, N.L., Burke, T., Rice, K.C., Pert, A. and Pert, C.B. The pattern of $[\text{}^3\text{H}]\text{cyclofoxy}$ retention in rat brain after in vivo injection corresponds to the in vitro opiate receptor distribution. Brain Res., 402: 275-286, 1986.
2. Ostrowski, N.L., Hill, J.M., Pert, C.B. and Pert, A. Autoradiographic visualization of sex difference in the pattern and density of opiate receptors. Brain Res., 421, 1-13, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02183-05 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Is Schizophrenia an Autoimmune Neuropeptide Receptor Disease?

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. B. Pert Guest Researcher NS, NIMH
Others: J. G. Knight Guest Researcher NS, NIMH
P. Laing Visiting Associate NS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

4.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The notion that schizophrenia has an important autoimmune component has been around for several decades, but has not previously been subjected to analysis by the most sensitive, modern techniques. We have hypothesized that early (perinatal) tolerance to viruses, which use the receptor molecule to gain cellular entry, causes this autoimmunity. We have developed a simple sensitive assay for detecting antibodies directed against human brain found in sera of schizophrenic patients and controls. We are now subjecting human brain membrane receptors to either native or denaturing solubilization conditions and separation by polyacrylamide gel electrophoresis (PAGE). After visualization by antiserum or CSF from patients and controls, we hope to identify the specific brain antigens against which schizophrenics have generated antibodies.

Project Description:Objectives:

To develop a simple assay for demonstrating brain-directed autoantibodies in schizophrenic sera; to demonstrate the molecular properties of these brain antigens and their distribution in brain tissue; and to explore the possibility that the antigens are cell surface neuropeptide receptors which mediate the biochemistry of emotion.

Methods Employed:

A novel filtration and centrifugation assay for detecting brain antigens in sera and the (McLean, et al., *Brain Res.* 278: 255-257, 1983) method for visualizing antibody distribution patterns in brain.

Major Findings:

In collaboration with Dr. Weber, over twenty experiments were performed for the purpose of optimizing the conditions of the antibody detection assay. In an early blind experiment, six of the Clinical Center 4-East ward acute schizophrenics' and controls' sera were examined. The two highest numbers in the assay belonged to the two sickest patients. We utilized the sera from these two patients vs. two controls in every experiment as we worked on optimization. The assay appears to sensitively and repeatedly demonstrate differences between controls and sera from other patients which were screened by Dr. DeLisi. The new patient's serum level appeared elevated even after repeated blood sample withdrawals over a period of one year. We know history of this area and are proceeding caustiously.

A significant (14%) percentage of hospitalized chronic patients have antibrain antibodies titers outside the control range.

A pilot study suggests macrophage chemotaxis is altered in schizophrenics.

Significance to Biomedical Research and Program of the Institute:

Schizophrenia is a crippling psychiatric disease which affects one percent of the general population. A complete, convincing understanding of its etiology would almost certainly lead to better therapeutic strategies and would place this psychiatric illness in a more "normal" context with other diseases of the body.

Proposed Course:

We must now collect a large number of determinations on many sera to describe the incidence in normal and schizophrenic sera as well as correlating psychotic symptoms with antibody titers over time within one patient. We plan to perform appropriate controls for neuroleptic drug treatment. We plan future experiments to test whether the incidence of antibodies directed against brain are much higher in schizophrenics--and perhaps certain subtypes--than in normal controls,

and that this incidence is not due to neuroleptic drug treatment. If schizophrenia is indeed a virally-triggered autoimmune disease, we will prove it and provide replicatable methods for others.

Publications:

1. Knight, J.G., Knight, A. and Pert, C.B. Is schizophrenia a virally-triggered anti-receptor autoimmune disease? In: Biological Perspectives in Schizophrenia, Helmchen, H. and Henn, F.A. (Eds.), John Wiley and Sons Ltd., New York, 1987, pp. 107-124.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02189-04 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropeptides and their Receptors are Shared by the Brain and the Immune System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. B. Pert Guest Researcher NS, NIMH

Others: P. Sacerdote Visiting Fellow NS, NIMH
 J. M. Hill Senior Staff Fellow NS, NIMH
 M. R. Ruff Guest Researcher NS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

2.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Neuropeptides, small signal peptides largely known for their role as transmitters of nerve impulses in the brain which mediate mood and emotion, have now been shown to regulate immune system function. Our work reveals that human monocytes have receptors and will respond chemotactically to numerous neuropeptides. Neuropeptides which we have reported on include β -endorphin and other opiates, substance P and bombesin. We have shown that a major class of psychoactive drugs, the benzodiazepines, are also potent chemoattractants. In this case we have directly demonstrated the presence of chemotactic receptors through ligand binding experiments. The presence of diverse, distinct neuropeptide chemotactic receptors on monocytes and other immune system cells suggests the existence of a neuroendocrine link between the brain and the immune system whose purpose is to integrate behavioral and emotional responses with immune system function.

In addition to the presence of neuropeptide receptors we have also been able to demonstrate that human alveolar macrophages store and secrete the neuropeptide bombesin. Neuropeptide synthesis is, therefore, a general feature of various immune cell populations. Such results are consistent with a multi-directional communication network via neuropeptides and their receptors. The purpose of this network is to link the body's cellular defense and repair systems with the nervous and endocrine systems and thereby integrate the internal milieu of the whole organism. The flow of information in this network is perceived by the human organisms emotional and/or altered states of consciousness. Ultimately, this results in behavioral decisions at the whole organism level. Additional work has suggested that a major cause of human cancer, small cell lung carcinoma, may not, as previously thought, arise from lung epithelium but originates from hemopoietic cells when the normal macrophage mediated repair of lung tissue is deranged by continuous heavy smoking.

Project Description:Objectives:

Neuropeptide effects on immune function: All considerations of health and well-being must necessarily attempt to understand immune system function within the context of the whole organism. For this reason we have considered the role of a class of molecules termed neuropeptides - short chains of amino acids primarily known for their role in nervous system function. What is becoming abundantly clear however is that these compounds, far from acting solely within the confines of the brain, are ubiquitous and have pleiotropic effects, functioning as transmitters, growth hormones, and signal agents for all the body systems. We feel that the neuropeptides are key components of a network whose purpose is to integrate behavior and brain function with immunological and endocrine systems.

In order to establish these points we have decided initially to examine the effects of neuropeptides in several ongoing model systems which deal with the immunological aspects of inflammatory responses, specifically the macrophage/granulocyte component. Macrophages figure prominently in many inflammatory processes, such as arthritis or gingivitis. Inflammation is known to have a neurogenic component, and neuropeptides (such as substance P), released from local nerves, appears to play a role in some inflammatory vascular reactions, as well as in arthritis. We questioned whether such locally released neuropeptides could exert some of their effects through immune cells such as the macrophage.

Our first objective was, therefore, to demonstrate a direct role for select neuropeptides in immune function. Additionally, we wished to consider the possibility that neuropeptides, as a general class of compounds, might have effects on immune function. Sporadic reports of neuropeptide effects on in vitro mast cell and lymphocyte function have been made over the last 10 years, however, no unifying concepts from this diverse (sparse) literature have emerged.

Small cell lung cancer as a disease of macrophages: Lung cancer is the leading cause of cancer death in the United States and greater than 25,000 people die each year of a subtype of lung cancer known as small cell (oat cell) lung cancer (SCLC). We have recently proposed that SCLC arises not from lung epithelium but rather from the macrophages present in the lungs of chronic smokers. Our interpretation of the etiology of SCLC emphasizes lung emphysema, inflammation, and tissue damage as a stimulus for myelopoiesis and recruitment of bone marrow derived macrophages into diseased lung. These cells then becomes transformed and give rise to the disease known as SCLC.

Our program is directed toward exploring similarities between SCLC cells and macrophages with the intent to develop novel therapeutic modalities. These studies will also focus on new aspects of inflammatory cell biology particularly the conditions which may lead to cell transformation and progression towards overt neoplastic disease.

Methods Employed:

Currently, we are testing various neuropeptides in several standard assays of macrophage function. This primarily involves an evaluation of the ability of the peptides to induce directed migration of macrophages and other cell types in Boyden chamber type assays. Many of the known chemotactic agents have additional effects on cell physiology and as active chemotactic peptides are identified they can be evaluated for other actions.

In addition to the functional assay of chemotaxis we have developed radioreceptor binding assays for one of the chemotactically active ligands we have studied (benzodiazepines) and are developing assays for others. These studies will permit a biochemical identification and, eventually, characterization of the receptors for some of these compounds. To date no neuropeptide receptors have been isolated in any system and binding studies (in some cases without any ascribed function) are only now being attempted on immune cells. In concert with these binding studies we are also attempting a preliminary biochemical characterization of one of the neuropeptide receptors we have identified on human monocytes (opiate receptor) through the method of chemical cross-linking of a radiolabeled ligand to its receptor.

We are examining immune cells for their possible content of several neuropeptides. Cell extracts are resolved by HPLC fractionation and peptides identified by radioimmunoassay. Specific anti-peptide antibodies are being used as histochemical probes to detect neuropeptides in specific immune cells and tissues.

Major Findings:

The opiate peptides (e.g., enkephalin, β -endorphin) are potent chemoattractants for human monocytes. Pharmacologic specificity can be demonstrated through the use of various agonists and antagonists of the opiate receptor. These compounds are exceedingly potent; activity can be detected at 10^{-14} concentrations. Several other neuropeptides have been found to have chemotactic activity for human monocytes with similarly low active concentrations. These include the hypotensive, bradykinin like peptide substance P, and the hypothalamic peptide bombesin. Pharmacologic studies using closely related analogs of these peptides indicate that chemotactic effects are mediated by specific neuropeptide receptors.

The benzodiazepine (e.g., valium) class of drugs are also potent chemoattractants for human monocytes. Binding studies confirm the presence of receptors with the appropriate structure/function relationship. Benzodiazepines are among the most widely prescribed drugs in the USA and no effects on macrophages or immune function have previously been described. The endogenous ligand for this receptor is a neuropeptide, only recently identified.

In addition to studies which have revealed a role for neuropeptides in monocyte chemotaxis we have also been able to show that other cells also express highly specific chemotactic receptors for these compounds. Thus, tumor cells, which may also express migratory potential, will chemotax in response to selected

neuropeptides. This response is not characteristic of all tumor cells but shows selectivity, both with respect to cells which respond and to peptides which are active. We have reported on the ability of neuropeptides such as bombesin, β -endorphin, substance P, and other to promote SCLC chemotaxis. Ongoing studies reveal that other highly metastatic tumors (e.g., breast) will also respond chemotactically to select neuropeptides. We have also shown that human spermatozoa will migrate chemotactically to various peptides. This new methodology, modified from our monocyte techniques, can now be utilized by researchers in human fertility who have to date not been able to assess sperm motility in any sensitive, quantitative fashion.

Small cell lung cancer: The cell of origin for SCLC has been speculative for 50 years but has focused on a neuropeptide secreting cell sparsely distributed in lung epithelium. Primarily, this is because SCLC cells synthesize a number of neuropeptides; most consistently bombesin. Our recognition of the importance of macrophages in inflammatory diseases and our studies on neuropeptide synthesis by macrophages prompted a direct test of the hypothesis that the putative neuropeptide secreting precursor was not a lung epithelial cell but another cell which figured prominently in the pathology of smokers lung, the macrophage. We were able to demonstrate four surface antigens, found only on macrophages and their precursors, to be present on cell lines and tumors of SCLC. These results are now confirmed and extended by other groups and we have interpreted these results to support our suggestion of a macrophage derivation for SCLC.

With regard to our studies on lung cancer it should be noted that no effective therapy exists for this disease and it is rapidly lethal; average life expectancy for untreated SCLC is 5-8 weeks. Oral cancer, although less common than lung cancer, is also associated with the use of tobacco products, and the etiology we propose for lung cancer is quite relevant to this disease as well. Our results have suggested a novel interpretation of the etiology of SCLC which suggests a number of unexplored therapeutic strategems.

The cell of origin for SCLC has been speculative for 50 years but has focused on a neuropeptide secreting cell sparsely distributed in lung epithelium. Primarily, this is because SCLC cells synthesize a number of neuropeptides; most consistently bombesin. Our recognition of the importance of macrophages in inflammatory diseases and our studies on neuropeptide synthesis by macrophages prompted a direct test of the hypothesis that the putative neuropeptide secreting precursor was not a lung epithelial cell but another cell which figured prominently in the pathology of smokers lung, the macrophage. We were able to demonstrate four surface antigens, found only on macrophages and their precursors, to be present on cell lines and tumors of SCLC. These results are now confirmed and extended by other groups and we have interpreted these results to support our suggestion of a macrophage derivation for SCLC.

Among the growth regulating hormones which control monocyte growth and differentiation are the interferons and colony stimulating factors. These and other immune hormones can now be evaluated systematically within this new conceptual framework for their effect on SCLC cells. The recent cloning of both of these hormones makes this an attractive approach as ample precedent documents the

ability of these agents to modify leukemia cell growth in some settings. Various combined modalities may ultimately prove more efficacious than current treatments; limited to chemotherapy.

Significance to Biomedical Research and Program of the Institute:

We have identified a group of very potent compounds, the neuropeptides, which exert hormone effects on human monocytes and other cells. Our results, primarily utilizing a chemotactic assay system are novel and indicative of a broader role for these class of compounds in immune system function.

Neuropeptides are known to cause both mood and behavioral alterations when acting within the brain, and to be released into the body during various emotional and physical states. Because these same peptides have very potent effects on macrophages, as well as other components of the immune system, we feel that these compounds are a major class of biochemicals which subserve information exchange between the brain and the body. The functional interaction of the body's cells through networks of neuropeptides and their receptors would be expected to be critical to the health of the organism as a whole and suggests a mechanism by which emotional states can significantly alter the course and outcome of biological illnesses previously considered to be strictly in the somatic realm.

The physiological correlate of *in vitro* monocyte chemotaxis to these chemicals is still obscure but it seems likely that the local release of neuropeptides may have important effects on cell distribution and activities. Thus, to cite one example, the peptide substance P has been implicated in the vascular erythematous reactions associated with inflammation and this peptide has very recently been shown to exacerbate experimental arthritis. Monocytes and lymphocytes (which also have substance P receptors) are prominent, locally present, cells which are primarily responsible for the degenerative changes which characterize arthritic lesions. Thus, it seems likely that this neuropeptide, by virtue of its ability to localise and activate immune cells may have an important causative role in this and other inflammatory processes. The recent demonstration that depletion of substance P from the local area surrounding an arthritic joint resulted in a substantial amelioration of the disease suggests the feasibility and importance of a program directed toward the understanding of neuropeptide effects on macrophage and immune function.

The ability of neuropeptides to effect monocyte and some tumor cell migration suggests a further role for these agents in histogenesis and tissue organization, serving to recruit and/or maintain resident macrophage and other cell populations. Disseminated neoplastic diseases may, to some extent, develop as a result of neuropeptide regulated cell trafficking. Thus, tumor cells, which have detached from the primary mass, may respond to organ (site) specific neuropeptide attractants. An understanding of this process could be relevant to controlling tumor spread and may help explain the frequent metastasis of some tumors (e.g., SCLC, breast) to neuropeptide-rich body sites.

Proposed Course:

Explore clinical settings in which neuropeptide macrophage mediated responses may have significant causative or diagnostic potential. Various systemic diseases with an underlying neuropeptidergic component may be reflected in altered macrophage neuropeptide chemotactic responses. An initial survey is being made of illnesses in which macrophages have a role, such as lung cancer to detect such alterations. Other diseases or conditions in which neuropeptides are known to play a role may also reveal alterations at the level of altered macrophage neuropeptide responsiveness. Neuropeptide therapy may prove useful in select illnesses by virtue of effects on macrophage or immune function. In vitro systems, such as chemotaxis, could be used to facilitate design of new drugs.

Establish the in vivo role for neuropeptides in macrophage function. Neuropeptides can be stimulated to be released at various sites (e.g., electrically, mechanically, chemically) and the accumulation of immune cells studied. These studies would support in vitro observations and could suggest the context in which physiological responses may occur. Such information could be important in understanding certain pathological states where macrophages accumulate and in devising ways to regulate their function.

Extended studies revealing antigenic similarities between macrophages and SCLC cells to explore functional similarities. This work will be directed at developing strategems for indutumoring cell differentiation and growth cessation with the aim of developing new therapeutic strategems. These studies will focus on possible lymphokine and monokine regulation of tumor growth/differentiation. We will also attempt to define the conditions which may result in transformation of inflammatory macrophages into cancer.

Biochemical studies aimed at characterizing neuropeptide receptors on monocytes and other immune cells. We will focus on receptor identification through binding and cross-linking studies with the aim of purification and raising antibodies. These studies will make it possible to examine the mechanisms of receptor function leading to cellular activation. Anti-receptor antibodies with agonist or antagonist activity could be used experimentally and possibly therapeutically.

Publications:

1. Wiedermann, C.J., Goldman, M.E., Sertl, K., Plutchok, J.J., Kaliner, M.A., Johnston-Early, A., Cohen, M.H., Ruff, M.R. and Pert, C.B. Bombesin in human and guinea pig alveolar macrophages. J. Immunol., 137: 3928-3932, 1986.
2. Wiedermann, C.J., Sertl, K. and Pert, C.B. Substance P receptors in rat spleen: characterization and autoradiographic distribution. Blood, 68: 1398-1401, 1986.

3. Wiedermann, C.J., Goldman, M.E. and Pert, C.B. Immunoreactive gastrin-releasing peptide in heat inactivated feta calf serum. Cell and Tissue Kinetics, 19: 467-470, 1986.
4. Gnessi, L., Fabbri, A., Silvestroni, L., Moretti, C., Fraioli, F., Pert, C.B. and Isidori, A. Evidence for the presence of specific receptors for N-formyl chemotactic peptides on human spermatozoa. J. Clin. Endocrinol. Metab., 63: 841-846, 1986.
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7. Hill, J.M., Lesniak, M.A., Rojeski, M., Pert, C.B. and Roth, J. Receptors for insulin receptor peptides in the CNS: studies of localization in rat brain. In: Insulin Structure and Function, Raizada, M. (Ed.), Plenum Press, New York, 1987, pp. 261-267.
8. Farrar, W.L., Hill, J.M., Ruff, M.R. and Pert, C.B. Visualization and characterization of interleukin 1 receptor in brain. J. Immunol., 139: 459-463, 1987.
9. Farrar, W.L., Hill, J.M., Ruff, M.R. and Pert, C.B. Visualization of cytokine and virus receptors common to the immune and central nervous systems. Lymphokine Res., 6: 29-34, 1987.
10. Wiedermann, C.J., Sertl, K., and Pert, C.B. Neuropeptides and the immune system: substance P receptors in bronchus-associated lymphoid tissue of rat. Annals of NY Acad. of Sci., in press.
11. Sacerdote, P., Ruff, M.R. and Pert, C.B. Cholecystokinin and the immune system: receptor-mediated chemotaxis of human and rat monocytes. Peptides, in press.
12. Wiedermann, C.J., Sertl, K., Zipser, B., Hill, J.M. and Pert, C.B. Vasoactive intestinal peptide receptors in rat spleen and brain: a shared communication network. Peptides, in press.
13. Hill, J.M., Lesniak, M.A. and Pert, C.B. Co-localization of IGF-II receptors, IL-1 receptors and Thy 1.1 in rat brain. Peptides, in press.
14. Ruff, M.R., Sacerdote, P., Wiedermann, C.J. and Pert, C.B. Neuropeptide receptors are shared components of nervous and immune systems. In: Neuropeptides and Stress, Tache, Y. (Ed.), Springer-Verlag, in press.
15. Wiedermann, C.J., Jelesoff, N.E., Pert, C.B. and Hill, J.M. Distribution of epidermal growth factor receptors in rat brain. Peptides, in press.

16. Weber, R.J., Hill, J.M. and Pert, C.B. Regional distribution of Thy 1.1 in rat brain. J. Neuroimmunol., in press.
17. Hill, J.M. Neuropeptides and their receptors as the biochemicals of emotions. In: Coping with Uncertainty: Biological Behavioral and Developmental Perspectives, Palermo, D.S. (Ed.), Lawrence Erlbaum Associates, Inc., New Jersey, in press.
18. Hill, J.M. and Pert, C.B. Neurochemical basis of emotional behavior. In: Handbook of Neuropsychology, Balber, F. and Grafman, J. (Eds.), Elsevier, Amsterdam, in press.
19. Hill, J.M., Lesniak, M.A., Kiess, W. and Nissley, S.P. Radioimmuno-histochemical localization of type II IGF receptors in rat brain. Peptides, in press.
20. Sacerdote, P., Wiedermann, C.J., Wahl, L.M., Pert, C.B. and Ruff, M.R. Visualization and characterization of cholecystokinin receptors on a subset of human monocytes and in rat spleen. J. Exper. Med., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02190-04 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Distribution and Properties of Opiate and Other Brain Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. B. Pert	Guest Researcher	NS, NIMH
Others:	M. R. Ruff	Guest Researcher	NS, NIMH
	C. M. Fraser	Pharmacologist	LNP, NINCDS
	C. J. Venter	Chief, Recept. Biochem.	LNP, NINCDS

COOPERATING UNITS (if any)

Section on Receptor Biochemistry, Laboratory of Neurophysiology, NINCDS

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

2.5

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cross-linking is a relatively recent biochemical strategy for covalently affixing reversible ligands to their recognition molecules for subsequent electrophoretic analysis. [¹²⁵I]-Tyr²⁷- β -endorphin (prepared as originally described by Smythe and co-workers) binds stereospecifically to rat brain membranes. While some studies have suggested that the β -endorphin receptor is a unique "epsilon" opiate receptor, a larger body of evidence suggest that β -endorphin has high affinity for most if not all of the opiate receptors types and subtypes. Cross-linking opiate receptors from different tissue sources can potentially reveal much information about the molecular basis of apparent opiate receptor heterogeneity. Cross-linking, however, only fixes 1% of the bound trace and SDS-PAGE while exquisitely sensitive, can fail to reveal substantial inter-molecular differences. Cross-linking was performed with the homo bi-functional reagent Disuccinimidyl Suberate (DSS). The iodinated cross-linking products of Tetrahymena, leech CNS, and rat brain membranes (both type 1 and type 2 conditions) appeared indistinguishable on SDS-PAGE gel with major cross-linking products at 58K and 100-110K. The strong cross-linked bands produced by incubation in the presence of the inactive opiate ((+)-naloxone) was completely abolished by the same (10⁻⁶M) concentration of its active isomer (-)-naloxone. Although we have thus far failed to distinguish between opiate receptors from a mammal, an invertebrate, and a unicellular organism, we continue to explore various conditions of binding, and electrophoresis, (e.g., reduced and unreduced) to examine possible receptor differences, both intra and inter species. Electrophoresis of proteolytic digests of cross-linked bands will be performed as a particularly sensitive method for distinguishing heterogeneity. Thus far, our cross-linking experiment suggest that the recognition molecule (the opiate receptor) which binds all opiate alkaloids and peptides is stable across evolution. As proposed, apparent physiological receptor heterogeneity is due to coupling to other membrane components.

Project Findings:

Objectives:

To map the neuroanatomical distribution of various chemically coded pathway in brain and to understand the neuroscientific significance of "multiple" receptors.

Methods Employed:

Newly developed in vitro autoradiography - unfixed frozen brain tissue is melted onto slides, incubated in appropriate radioactive ligand to label receptors, washed serially, dried rapidly, fixed with paraformaldehyde vapors and dipped in radiosensitive liquid emulsion for autoradiographic visualization.

Sophisticated computer analysis of receptor binding kinetics is used to rigorously define conditions of multiple opiate receptor binding.

For the first time we bring together rigorous kinetic analysis with autoradiographic distribution of binding sites.

We are cross-linking reversible ligands covalently to their recognition molecule for subsequent electrophoretic analysis.

Major Findings:

One opiate delta receptor appears conformationally fixed, while the other appears capable of assuming mu, delta and kappa conformations.

We showed that β -endorphin labeled opiate receptor from rat, leech and Tetrahymena have the same molecular weights of 58 and 110Kd on SDS-PAGE. This suggests that the opiate receptor is stable across evolution.

Significance to Biomedical Research and Program of the Institute:

Pinpointing neurochemically coded tracts will enable us to determine the functional significance of each newly discovered pathway. The method can be used on human brain and ultimately should give information about the contribution of various neurochemically coded tracts to pathology.

Proposed Course:

We plan a sophisticated biochemical and immunological approach to further defining the molecular nature of opiate receptors. The type 1 opiate receptor complex with its advanced evolutionary accumulation in the forebrain of humans seems particularly worthy of further study (see Project Number Z01 MH 02182-03 NS, Toward the Visualization of Opiate Receptors in Living Human). We plan to study the brain distribution of insulin, transferrin, and their receptors to further demonstrate the breakdown in the distinction between "neuropeptides" and hormones.

Publications:

1. O'Neill, J.B., Pert, C.B., Ruff, M.R., Smith, C.C., Higgins, W.J. and Zipser, B. Identification and characterization of the opiate receptor in the ciliated protozoan, Tetrahymena. Brain Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02191-02 NS

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Receptors for the AIDS Virus and Other Neurotrophic Viruses

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. B. Pert Guest Researcher NS, NIMH

Others: J. M. Hill Senior Staff Fellow NS, NIMH
 E. M. Sternberg Guest Researcher NS, NIMH
 M. R. Ruff Guest Researcher NS, NIMH
 P. Sacerdote Visiting Fellow NS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We have demonstrated that the 60 kD protein previously characterized on a subset of T lymphocytes and named "T4", is another example of shared components between the brain and immune system. Thus, we have demonstrated that this cell surface molecule can be cross-linked to ¹²⁵I labeled AIDS virus envelope and immunoprecipitated by the Mab OKT4 in both T cells and brain. Furthermore, we have mapped the brain distribution pattern of the AIDS virus receptor, T4 in monkey, human and rat brain and shown that it is most enriched in areas of the cortex and the hippocampus which subserves cognition and other higher functions. Our work suggest that the neuropsychiatric effects of AIDS may not, as previously thought, be due to inflammatory processes but may be due to a direct neuronal infection of the virus.

We have identified, synthesized, and studied an octapeptide "peptide T", which appears to be the critical attachment area of the AIDS viral envelope. Peptide T and several rationally designed peptide analogs appear to bind with high affinity to the AIDS virus receptor, blocking viral infectivity at very low concentrations. We expect that synthetic peptide heteropolymers employing this core pentapeptide attachment sequence will prove valuable as an approach for a vaccine for AIDS.

This method and approach appears useful for exploring the presence of other virus receptors in brain. For example, we have already observed that the Epstein-Barr virus which has been known to use the complement receptor on B cells as a receptor entry protein, may actually infect brain via the same receptor molecule which we have recently identified in brain.

Project Description:Objectives:

To find the cure for AIDS.

Methods Employed:

Virus receptor binding, viral infectivity, human monocyte chemotaxis, cross-linking to and molecular separation of brain and immune cell receptors.

Major Findings:

By molecular characterization and immunoprecipitation, we have demonstrated that the AIDS virus receptor (T4) is present in human, monkey and rat brain in an indistinguishable form as that present on human T cells. We are isolating an endogenous peptide ligand that binds to these receptors, which will presumably mediate behavioral activity as well as immune function, from peptide extracts of rat brain. Meanwhile, synthetic peptides have been deduced with computer-assistance which bind with very high affinity (10^{-11} M) to AIDS virus receptors on rat brain membranes and displace radiolabeled viral envelope protein (gp120) at the same low concentrations. A series of peptide analogs have been constructed and a structure activity relationship for T4 receptors has been documented. This structure activity relationship appears to be constant whether human monocyte chemotaxis, AIDS virus infectivity, behavioral activity of rats after intraventricular injection, displacement of radiolabeled AIDS viral envelope, or inhibition of lymphocyte PHA mitogenesis or DR expression is studied.

Clearly the AIDS virus receptor and its endogenous peptide ligand are yet another example of a neuropeptide receptor and ligand subserving intercellular communication throughout the brain and body.

Significance to Biomedical Research and Program of the Institute:

AIDS is the #1 public health problem in the USA. Unexpectedly, an understanding of neuropeptides and their receptors, a specialty of our Institute's Program in general and my research specifically, is highly desirable to understand AIDS.

Proposed Course:

We will optimize peptide structure to prevent proteolysis and thus obtain "The Ultimate Peptide". We will use knowledge gained from this viral disorder to understand schizophrenia.

Publications:

1. Hill, J.M., Farrar, W.L. and Pert, C.B. Localization of the T4 antigen/AIDS virus receptor in monkey and rat brain: prominence in cortical regions. Psychopharmacology Bulletin, 22: 686-694, 1986.

2. Hill, J.M., Farrar, W.L. and Pert, C.B. Autoradiographic localization of T4 antigen, the HIV receptor, in human brain. Intl. J. Neurosci., 29: 687-693, 1986.
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14. Sternberg, E.M., Damschroder-Williams, P.J., Weber, R.J. and Pert, C.B. Peptide T, VIP and their analogs induce Ia expression in murine macrophages. Proc. Natl. Acad. Sci. USA, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 201 MH 02152-08 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Discipline and Parental Control in Families with Affective Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. Kochanska	Research Psychologist	LDP/NIMH
Other:	L. Kuczynski	Assoc. Prof. of Psychology	Univ. of Guelph Guelph, Ontario
	M. Radke-Yarrow	Chief	LDP/NIMH

COOPERATING UNITS (if any)

University of Guelph
 Guelph, Ontario Canada

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.82

PROFESSIONAL:

.35

OTHER:

.47

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Mothers' discipline and control practices and their children's responses to maternal control attempts are studied in well and clinically depressed mothers. Impaired parental skills in managing children's behavior have often been implied in the etiology of maladaptive patterns of child development. Depressive symptomatology has been linked to inappropriate control practices, but specific difficulties of depressed mothers and possible implications for their children's development have not been identified. Assessments of mother and child behavior are based on observations of their interaction in a naturalistic setting (see Annual Report MH 02144). Detailed measures of maternal control were taken, including goals, timing and specific techniques, and the overall interactive quality of control episodes. Child response was also measured in terms of compliance to maternal demands and in terms of level of social competence of non-compliance strategies. Analyses revealed that affectively ill mothers, compared to well mothers, have specific difficulties controlling their children. In particular, they are more likely to avoid confronting the child and less able to negotiate a compromise after their initial intervention meets with child's resistance. Children of well mothers, but not of depressed mothers, became more cooperative over time. Daughters of depressed mothers appeared at particular risk for noncompliance problems.

Project Description

The determinants, contents, and effects of parental discipline and control practices in families with normal and clinically depressed mothers are investigated. Effective, yet harmonious parental control of child behavior is crucial for child development and socialization. It becomes of particular importance in the second and third year of life when two conflicting developments occur: the child becomes able to regulate his/her own behavior and to comply with parental demands, but also becomes more overtly resistant towards caregivers, which is a manifestation of emerging autonomy. If the parent is not able to maintain effective control of the child, and at the same time to promote the child's emerging autonomy, this may be a source of future maladjustments and dysfunctions. Maternal depression has often been associated with general maladaptive patterns of control, such as hostility, punitiveness, and low involvement. However, more specific processes have not been identified. Also, the impact of maladaptive maternal strategies on the development of the child has not been determined.

Mothers' control practices were examined in terms of their effectiveness in obtaining child compliance, and in terms of promoting acceptable and competent forms of ascertaining child autonomy.

Methods

The basic paradigm is described in Annual Report # MH-02144. Control interventions of 33 well, 37 unipolar depressed and 17 bipolar depressed mothers and their children (16 to 50 months of age) were analyzed. Each control episode occurring during 90 minutes of naturalistic interactions was coded, starting with the mother's attempt to regulate child behavior and continuing until the issue was resolved or dropped. Assessments included: timing of the intervention, specific goal, and verbal, affective and physical components of maternal intervention. The child's response was coded in terms of compliance or noncompliance. In our measures of noncompliance we tried to capture the child's developing level of competence, revealed in his/her resistance strategies. We distinguished less competent forms: passive noncompliance and overt defiance; direct, but nonaversive refusals; and most developmentally advanced, indirect and nonaversive attempts to bargain or modify parental demands (negotiation). Each episode was also coded for its resolution, capturing interactive qualities and final outcome. Categories included: immediate maternal success, ultimate maternal success by persuasion, ultimate maternal success by enforcement, non-confrontation, ultimate maternal failure, compromise and unresolvable episodes.

Findings

Analyses revealed that maternal psychopathology affected the pattern of control interactions between mothers and their toddlers. The affectively ill women, more than the well women, avoided confrontation with their children when faced with their resistance; they also had more troubles reaching a compromise with their children. Slightly different patterns of impairments appeared for severely ill uni- and bipolar mothers.

The daughters of depressed mothers seemed most at risk for noncompliance problems. In addition to the findings related to maternal psychopathology, the study gives some insight into the developmental nature of child opposition to socialization pressure. More competent forms of resistance (negotiation) were found to increase between the second and third year of life. Less socially skillful forms (direct defiance, passive noncompliance) decreased over time. The level of social competence of child resistance strategies was found to be related to the nature of influence techniques used by the mother.

Significance to Biomedical Research

Children of depressed parents are at greater risk for psychopathology and behavioral disorders than are children of normal parents. Research on child development has demonstrated that aberrant parental disciplinary practices are important contributors to children's disordered social and emotional development. How depression affects the parent's abilities to function in controlling child behavior is largely unresearched; yet this variable may contribute significantly in creating a pathogenic environment for young children.

Proposed Course

Three manuscripts have been submitted to journals. At present, work is focused on more detailed analysis of mothers' verbal and physical control strategies as related to their history of depression, as well as their current mood state. A manuscript is being prepared. Another focus is on mothers' socialization goals as expressed in their control interactions. Both developmental, and psychopathology issues will be addressed.

Publications

Kochanska, G., Kuczynski, L., Radke-Yarrow, M., and Friedman, S.: Normal and affectively ill mothers' beliefs about their children. Am. J. Orthopsychiatry, 57(3): 345-350, 1987.

Kochanska, G., Kuczynski, L., Radke-Yarrow, M., and Welsh, J.D.: Resolutions of control episodes between well and affectively ill mothers and their young children. J. Abnorm Child Psychol., in press.

Kuczynski, L., Kochanska, G., Radke-Yarrow, M., and Girnius-Brown, O.: A developmental interpretation of young children's noncompliance. Dev. Psy., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 MH 02155-08 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Children of Depressed and Normal Parents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. Zahn-Waxler	Chief, Sec. on	LDP/NIMH
		Child Behavior Disorders	
Other:	E. Cummings	Professor	University of West Virginia
	R. Iannotti	Research Psychologist	Georgetown University
	K. Rubin	Professor	University of Waterloo
	S. Denham	Associate Professor	George Mason Univ.

COOPERATING UNITS (if any)

University of West Virginia	George Mason University
University of Waterloo	
Georgetown University	

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Child Behavior Disorders

INSTITUTE AND LOCATION

National Institute of Mental Health, NIH, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS:

.82

PROFESSIONAL:

.40

OTHER:

.42

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is to identify early in development dimensions of personality and social-emotional functioning that relate to the expression of problem behaviors in children as they reach school age. The focus is on the development of both externalizing (aggression) and internalizing (anxiety and depression) symptoms. Patterns of continuity and discontinuity are examined separately for children from high and low risk environments. Children's emotion expressions and social interactions at age two and age five were observed. A psychiatric assessment was obtained at age six. Significant patterns of continuity in internalizing and externalizing problems have been identified; early problem behaviors observed in children's social interactions predict internalizing and externalizing symptoms at school age. Good social skills in toddlers appear to be protective factors. Further coding and analyses are in progress to assess children's appropriate use of affect in guiding social interactions, their strategies for achieving goals and solving problems in interpersonal situations, and the nature of their dyadic play.

Project Description

The purpose of this research is to identify early in development patterns of behavior that relate to the expression of problem behaviors in children as they reach school age. Internalizing symptoms or problems of overcontrol (depression, anxiety, social withdrawal) and externalizing disorders or problems of undercontrol (aggression, conduct problems) are investigated in a sample of six-year-old children. Patterns of continuity/discontinuity are examined separately in children from high and low risk environments. Risk is defined in terms of parental psychopathology (maternal depression), since this factor has been linked both with aggression and depression in children. Problem behaviors in six-year-olds are examined in relation to social competence and affective coping styles. Parental variables expected to influence aggression and depression in children are also assessed.

Methods Employed and Major Findings

Forty-eight two-year-old children were seen in three 1 1/2 hour laboratory sessions spaced two weeks apart. Children of both normal and depressed mothers (SADS-L) were studied. Each child was exposed to a range of challenging conditions in order to evaluate social and emotional interchanges primarily in interactions with a familiar playmate, but also with mother and with an adult stranger. Assessments were made of the child's ability or lack of ability to sustain social play, compete adaptively for resources, negotiate problems, cooperate, cope with frustration without resorting to intense aggression, empathize, and solve hypothetical social problems. Maternal characteristics evaluated were sensitivity, supportive presence and quality of assistance, and techniques used to encourage cooperation and sustained social and task-oriented involvement with others. Children's social skills in peer interactions were assessed again at age five. Self-report data on childrearing practices and the marital relationship were also obtained. At age six the Childhood Assessment Schedule, a psychiatric interview, was used to obtain psychiatric evaluations of the children. The mothers completed the Achenbach Child Behavior Check List.

Significant patterns of continuity in internalizing and externalizing problems were identified in preliminary data analyses and are detailed in last year's annual report: early problem behaviors observed in children's social interactions predicted problem behaviors at follow-up and good social skills in young children functioned as protective factors. This year's work has focused on the development of more differentiated and elaborated systems for assessing children's social skills and deficits in their interactions with others. Three dimensions of social competence that might be expected to predict internalizing and externalizing problems are examined: (1) the child's appropriate or inappropriate use of affect in guiding social interactions, (2) the child's strategies for achieving goals, expressing needs, and asserting rights in interpersonal situations, and (3) the quality and type of dyadic play patterns.

Proposed Course

The data have been coded, and data analyses continue. Three manuscripts are in preparation: developmental changes and patterns of individual differences in social competence in two- and five-year-old children, social problem-solving strategies and play patterns in five-year-old children of depressed and non-depressed mothers, and composites of factors early in development that predict diagnosable problem behaviors (internalizing and externalizing patterns) in six-year-old children.

Significance to Biomedical Research and the Program of the Institute

An aim of prevention and intervention research is to identify early in development those child and family factors that contribute to later childhood disturbances. Childhood depression and antisocial behavior have tended to come to the attention of professionals when children reach school age, but they may have much earlier origins. If early identification of behavior problems can be made, more effective intervention procedures could be planned. This is a final report.

Publications

Pierrehumbert, B., Iannotti, R., Cummings, E., & Zahn-Waxler, C. Attachement maternal et dependance: Quelques apports de la psychologie experimentale. Neuropsychiatrie de l'Enfance, 1986, 34(8-9), 409-420.

Friedman, S.L., Zahn-Waxler, C., Waxler, M., & Werthmann, M. Effects of physiologic jaundice on behavioral function in low risk pre-term infants. J. Appl. Dev. Psychol., 8: 53-66, 1987.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02156-08 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Personality of Children Reared by Normal and Depressed Mothers: Inhibited Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F. Bridges-Cline Guest Researcher LDP/NIMH

Other: G. Kochanska Research Psychologist LDP/NIMH

COOPERATING UNITS (# any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.22

PROFESSIONAL:

.10

OTHER:

.12

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study focuses on the role of early childhood inhibition in the development of pathological risk indicators in children of families with and without parental depression. Patterns of child behavior in the face of the unfamiliar (persons, places) such as behaviors expressing inhibited exploratory activity and social withdrawal are observed at 2 to 3 years of age in semi-naturalistic but standard settings, which represent varied contexts of unfamiliarity. Analyses of these data revealed that five reliable dimensions of response styles could be empirically derived from our observation coding system. These dimensions meaningfully distinguish groups of children in our sample at this very young age. Comparisons of these five dimensions of early behavioral inhibition across maternal diagnostic groups (normal, major depressive and bipolar) indicate that the young children of the major depressive mothers typically exhibit the most inhibited forms of exploratory behavior, a strong tendency to cling to mother in novel situations, and characteristically flat and withdrawn displays of affect. Children of the bipolar mothers typically exhibit the most active and independent forms of explorations of novel environments. In a situation of an unfamiliar person, these children score at both the inhibited and uninhibited polar extremes. However, in this situation, these children of bipolar mothers do not demonstrate a clinging dependency on mother and typically present positive displays of affect. Observational data relating to mothers' behaviors that are concurrent with child behaviors in these situations are being analyzed to examine the ways in which mothers in these groups function to facilitate or hinder the child's exploration and approach of unfamiliar situations.

Project Description:

Reduced motor activity, anhedonia, disinterest in activity, and social withdrawal are behavioral characteristics that are often associated with depression. However, the role of early behavioral forms of these characteristics as precursors to later manifestations of depression has not been established. One of the early forms of these characteristics is behavioral inhibition, as revealed by the way in which the child engages with novelty or unfamiliarity. One purpose of this study is to identify and describe the patterns of response to unfamiliarity that are exhibited by young children of depressed and nondepressed mothers. Another question of interest is how mothers' handling of their children in these situations may differ. Of predictive interest is the consequence of these early behavioral patterns for later manifestations of disordered behavior.

Methods Employed and Major Findings:

Patterns of response to unfamiliarity are studied in children of depressed and nondepressed mothers at 2 to 3 years of age. The children's behavior has been videotaped in varied situations of unfamiliarity, such as entrance to an unfamiliar but attractive environment, and introduction to and interaction with an unfamiliar but friendly adult. Child behavior measures that are coded from these videotapes include: latency measures, such as latency to touch objects or toys in the entrance situation, proximity to and reliance upon mother in order to interact or explore, and levels of exploration and interaction such as scanning and looking, or retreating from stranger in contrast to actively manipulating objects or initiating interaction with the unfamiliar adult. In addition, the way in which the mother functions to facilitate or hinder the child's approach and engagement of the unfamiliar is studied.

A factor analysis was performed. The five factors emerged: (1) Exploratory Activity, (2) Seeks Mother Support in Exploration (3) Initiating Engagement of Stranger (4) Responder-role in interaction with stranger, and (5) Flat and Withdrawn. A comparison of the distributions of these response style characteristics across the maternal diagnostic groups normal, bipolar, and major depressive also replicated the direction and nature of the earlier preliminary findings, indicating the robustness of these conclusions. Children of the major depressive mothers typically show more cautious, less active, less initiating, and more mother physical-dependency styles in both the entrance and stranger situations of unfamiliarity. And these children typically exhibit sad and/or flat affect, in conjunction with withdrawal from interaction with the stranger. Children of the bipolar mothers, on the other hand, are characteristically positive and animated in their affect displays, and, as a group, look very similar to one another in their active and mother-independent styles of exploring new environment. Further, although the children of the bipolar mothers do distribute themselves at both polar extremes of the Stranger Engagement scale, they, in contrast to the children of the major depressive mothers, show very little clinging to mother as a style of retreating and withdrawing from unfamiliar people.

Significance to Biomedical Research:

Our observational coding system focusing on the behavioral inhibition of the 2- to 3-year-old children in our sample yielded a set of stable and interpretable response style patterns, which meaningfully distinguish groups of children at this very young age. Accomplishment of these objectives--reliable assessment of the young child's behavioral expression of reduced activity and/or social withdrawal in the face of the unfamiliar--is a significant step toward a better understanding of the developmental course of these characteristics and of their link to later manifestations of depression or other disordered behavior.

Proposed Course:

A manuscript describing the response style patterns that emerged from these data and the differences between the children of the three maternal diagnostic groups is being prepared. In addition, further analyses are being conducted to examine the role of mother behavior in the developmental course of these child behavior patterns.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02164-07 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological changes and psychological functioning during adolescence

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.J. Susman	Guest Researcher	LDP NIMH
OTHER:	E.D. Nottelmann	Statistician	LDP NIMH
	G.I. Germain	Research Psychologist	LDP NIMH
	L.D. Dorn	Guest Researcher	LDP NIMH
	G.P. Chrousos	Senior Investigator	DEB NICHD
	G.G. Cutler	Senior Investigator	DEB NICHD
	D.L. Loriaux	Chief	DEB NICHD

COOPERATING UNITS (if any)

Developmental Endocrinology, NICHD

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.97

PROFESSIONAL:

.75

OTHER:

.22

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The interrelations of psychological functioning and endocrine and physical growth factors in young adolescents are investigated. Participants are 56 boys and 52 girls, 9 to 14 years old, and their parents. Participants were evaluated three times, six months apart on (a) stage of pubertal development (Tanner criteria), and hormone levels (serum levels of gonadotropins, gonadal steroids, adrenal androgens, and cortisol), and (b) psychological status (cognitive functioning, moods, problem behavior, competencies, social supports and interactions with parents). Higher levels of adrenal androgens and cortisol and older chronological age predicted higher degrees of self-reported anxiety and depressive symptoms for boys; for girls, these symptoms were predicted by lower levels of gonadotropins. For both sexes, adolescents who reported an absence of psychological support from their parents also reported higher degrees of anxiety and depressive symptoms. In a clinical situation involving a mild stress (blood drawn for hormone assessments), adolescents with higher cortisol levels exhibited higher levels of observed behavioral distress on first exposure to the stressor. This association disappeared when the situation became familiar.

Project Description

The interrelations of behavioral competencies and dysfunctions and endocrine and physical growth changes in young adolescents are examined cross-sectionally and longitudinally.

Methods and Findings:

The interrelations of psychological functioning and endocrine and physical growth factors in young adolescents are investigated. Participants are 56 boys and 52 girls, 9 to 14 years old, and their parents. Participants were evaluated three times six months apart on (a) stage of pubertal development (Tanner criteria), and hormone levels (serum levels of gonadotropins, gonadal steroids, adrenal androgens, and cortisol), and (b) psychological status (cognitive functioning, moods, problem behavior, competencies, social supports and interactions with parents). (The details of measurement are reported in previous Annual Reports.)

A general pattern of findings, for boys, is that higher levels of adrenal androgens and cortisol and higher age predicted more anxious and depressive symptoms one year later; for girls, lower levels of gonadotropins predicted anxious and depressive symptoms. For both sexes, the absence of psychological support from parents was associated with higher degrees of anxious and depressive symptoms.

Physiological reactivity and behavioral reactivity were examined in terms of adolescents' responses to a potentially stressful situation (blood drawn for hormone levels and a physical examination for pubertal staging). Cortisol level and changes in level were measured across a 40-minute period. Behavior was coded for complaints of pain, muscular rigidity, crying, and physical resistance. Adolescents with higher cortisol levels exhibited more distress behaviors than those with lower levels during the blood-drawing procedure at the first time of assessment. The association disappeared when the situation became familiar, i.e., on second and third visits. Individual differences in the pattern of change (increased, decreased, or no change) in cortisol level across forty minutes were similar over the one-year period.

Significance to Biomedical Research

Increases in behavior problems, psychiatric disorders, and suicides occur during adolescence. These behaviors are undoubtedly affected by multiple influences. One component of potential influence is the physiological changes that occur during this developmental period. Better knowledge of the interrelations among hormonal changes, physical growth changes, and family and peer interactions investigated in this study may contribute to the understanding of this period.

Proposed Course

Manuscripts are in press, submitted for publication, and in preparation. Coding of observational data and longitudinal analyses will continue over the next year.

Publications

Susman, E.J., Inoff-Germain, G., Nottelmann, E.D., Loriaux, D.L., Cutler, G.B., Jr., and Chrousos, G.P. Hormones, emotional dispositions and aggressive attributes in young adolescents. Child Dev. 58: 1114-1134, 1987.

Susman, E.J., Nottelmann, E.D., Inoff-Germain, G., Dorn, L.D., and Chrousos, G.P. Hormonal influences on aspects of psychological development during adolescence. J. Adolesc. Health Care. In press.

Susman, E.J., Nottelmann, E.D., Dorn, L.D., Inoff-Germain, G., and Chrousos, G.P. Physiological and behavioral aspects of stress in adolescence. In G.P. Chrousos (Ed.), Mechanisms of Stress. New York: Plenum Publishers. In press.

Susman, E.J., Dorn, L.D., and Fletcher, J.C. Reasoning about illness in ill and healthy children and adolescents: Cognitive and emotional developmental aspects. J. Dev. Beh. Pediatr. In press.

Trickett, P.K. and Susman, E.J. Perceived similarities and disagreements about child-rearing practices in abusive and nonabusive families: Inter-generational and concurrent family processes. In D. Cicchetti and V. Carlson (Eds.), Theoretical Perspectives and Research on the Consequences of Child Maltreatment. New York: Cambridge University Press. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02169-05 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Interactions Between Siblings With a Depressed Parent

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. Zahn-Waxler	Research Psychologist	LDP NIMH
OTHER:	D. Hay	Research Psychologist	Univ. of London
	M. Radke-Yarrow	Chief	LDP NIMH

COOPERATING UNITS (if any)

Univ. of London

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Child Behavior Disorders

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been incorporated into project Z01 MH 02370.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02170-05 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychiatric Assessment of Infants and Toddlers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	L. Cytryn	Medical Officer (Psych)	LDP/NIMH
Other:	T. Sherman	Research Psychologist	LDP/NIMH
	D. McKnew, Jr.	Medical Officer (Psych)	LDP/NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Affective Development

INSTITUTE AND LOCATION

National Institute of Mental Health, NIH, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS:

1.55

PROFESSIONAL:

.45

OTHER:

1.10

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One hundred and twenty-three children, 2 to 3 years of age, were assessed using two structured, behavioral observation systems: in a psychiatric play interview in the absence of mother, (see Z01 MH 02170-03), and in interaction with mother (see Z01 MH 02230-02). Twenty-two mothers had Bipolar Disorder, 45 Major Depressive Disorder, 11 Minor Depressive Disorder, and 45 with no history of psychiatric disorder. All children were assigned a rating (1 to 4) of degree of risk for later development of psychopathology based on their performance in each setting. This produced four groups of children: a low risk group with low risk ratings in both assessments, two mixed-risk groups with ratings of 1 or 2 in one setting, and 3 or 4 in the other, and a high risk group with ratings of 3 or 4 in both assessments. From a case-by-case analysis of the 16 children in the high risk group, three major constellations of behavioral symptoms were discernible: One was seen in a boy from a healthy home who displayed little affect, had a very distant type of relationship with his mother, and would not speak with the psychiatrist in the play session. A second pattern was seen in three girls from relatively poor families in which mother suffered from major depression. This pattern consisted of a distant type of relationship with mother, dysphoria, and anhedonia. The third pattern was seen in the remaining group of twelve children (4 girls and 8 boys). These children came from homes that varied in social class, and diagnostic status of the parents. The behavior consisted of an angry relationship with mother, and predominantly angry mood. The children in all three groups demonstrated dysregulation of emotions under stress, and little or no interest in play.

Project Description

The goal of this project is to develop a nosology of psychopathology for children aged two to four years of age. To this end we have scripted a series of behavioral episodes to reveal a wide range of the young child's behavioral repertoire as well as his modal pattern of behavioral functioning. Two behavioral observation systems that allow for reliable and systematic recording of the child's behavior are used. One instrument requires 30 to 40 minutes of observation of the child when separated from mother. This instrument can be used by the clinician as s/he interacts with the child. The second is used for evaluation of the child's interaction with mother. A decision tree model of the clinician's synthetic processing of his/her behavioral observations was used and a reasonable level of concordance was found between the ratings derived via the decision tree and the ratings provided by experienced clinicians. The details of the two behavioral observation systems, as well as the decision tree model of clinical synthesis were presented in reports (Z01 MH 02170-03 and Z01 MH 02230-02).

One hundred and twenty-three children, 2 to 3 years of age, were assessed using the two behavioral observation systems. Twenty-two mothers had Bipolar Disorder, 45 Major Depressive Disorder, 11 Minor Depressive Disorder, and 45 had no history of psychiatric disorder. Approximately one-half of the husbands of the mothers with affective disorder had affective disorder as well.

Methods and Major Findings

All children were assigned a rating (1 to 4) of degree of risk for the later development of psychopathology based on their performance in each of the settings. This produced four groups of children: a low risk group who received ratings of low risk for the later development of psychopathology in both assessments (60 children), two mixed-risk groups composed of children who received a rating of high risk in one setting and low risk in the other (12 children high risk with mother, low risk with the psychiatrist, 35 children low risk with mother, high risk with the psychiatrist), and a high risk group of children who received ratings of high risk in both assessments (16 children).

From case analyses of the 16 children in the high risk group, three major constellations of behavioral symptoms are discernible. One was seen in a boy from a healthy home who displayed little affect, had a very distant type of relationship with his mother, and would not speak with the psychiatrist in the play session. A second pattern was seen in three girls from relatively poor families in which mother suffered from major depression. It consisted of a distant type of relationship with mother, dysphoria, and anhedonia. The third pattern was seen in twelve children (4 girls and 8 boys). These children came from homes that varied in social class, and diagnostic status of the parents. The behavior consisted of an angry type of relationship with mother, and predominantly angry mood. The children in all three groups demonstrated dysregulation of emotions under stress, and little or no interest in play.

Significance to Biomedical Research

The significance of this research is multifold: The development of improved assessment instruments will help in understanding the developmental patterns of adaptation and maladaptation in very young children and will permit more sensitive evaluation of the children's strengths and vulnerabilities. These assessments enable us to see whether patterns of adaptation differentiate between children reared by normal and depressed mothers. We will be able to evaluate how assessments from this perspective compare with later standardized psychiatric assessments of the children, and see whether any of the patterns of adaptation identified in these young children predict to the later occurrence of specific psychopathologies. This prospective information not only adds to our understanding of the developmental course of affective illness, but may provide an informed basis for identifying children who are most at risk.

Proposed course:

Analyses will be completed, and manuscripts will be prepared for publication in a scientific journal.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02171-04 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Protective and Risk Factors in Childrearing: Contributions of Fathers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W.E. Wilson	Guest Researcher	DRG/NIH
OTHERS:	J.E. Richters	Staff Fellow	LDP/NIMH

COOPERATING UNITS (if any)

Division of Research Grants, NIH

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.70

PROFESSIONAL:

.25

OTHER:

.45

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The husbands of depressed women may buffer their children from the stresses of living with a depressed mother by encouraging dialogue, maintaining stability and equilibrium within the family, and providing children with a supportive environment. But they may also exacerbate the stresses to which their children are exposed by not functioning effectively in these roles.

The objective of the present study is to examine the functioning of spouses by combining measures of psychopathology and deviance in the spouses of depressed women with detailed assessments of their functioning with and around their wives and children. These measures will then be examined for their contributions to predictions of children's adjustment. Analyses will be based on interview, case history, and direct observation data from families participating in a follow-up phase of the NIMH Childrearing Project, which includes children of parents with and without a history of affective disorder.

Project Description

The husbands of depressed women may buffer their children from the stresses of living with a depressed mother by encouraging dialogue, maintaining stability and equilibrium within the family, and providing children with a supportive environment. But they may also exacerbate the stresses to which their children are exposed by not functioning effectively in these roles.

The objective of the present study is to examine the functioning of spouses by combining measures of psychopathology and deviance in the spouses of depressed women with detailed assessments of their functioning with and around their wives and children. These measures will then be examined for their contributions to predictions of children's adjustment.

Method

Data are from families participating in a follow-up phase of the NIH Childrearing Project (Z01 MH 02144), which includes children of parents with and without a history of affective disorder. Follow-up assessments include detailed psychiatric interviews with mothers, fathers, and children, interviews with fathers concerning their role in child-rearing and family functioning, and intensive interviews with mothers concerning their marriages and family life. In addition, fathers were videotaped while interacting with their children and wives -- separately and in combination -- in a series of naturalistic settings within our laboratory apartment.

Proposed Course

The coding and scoring of interviews is in progress. A system for assessing the father's role in family interaction based on our observational data is currently being developed. Following completion of data analyses in the Spring of 1988, manuscripts will be prepared and submitted for publication.

Significance to Biomedical Research

This study will advance our understanding of the link between deviance in the husbands of depressed women and elevated rates of risk for maladjustment and psychopathology among their offspring.

Publications

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02207-04 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Affective Rearing Environment: A Comparison of Normal and Depressed Parents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Radke Yarrow	Chief	LDP NIMH
OTHER:	G. Kochanska	Res. Psychologist	LDP NIMH
	W.E. Wilson, Jr.	Res. Psychologist	LDP NIMH
	L. Kuczynski	Assoc. Professor	U. Of Guelph
	E. Nottelmann	Statistician	LDP NIMH
	J. Richters	Res. Psychologist	LDP NIMH
	B. Belmont	Soc. Sci. Analyst (Clin.)	LDP NIMH
	A. Mayfield	Soc. Sci. Analyst	LDP NIMH
	J. Stilwell	Res. Nurse Prac. (Psychiatric)	LDP NIMH

COOPERATING UNITS (# any)

Univ. of Guelph, Guelph, Ontario, Canada
 Division of Research Grants, NIH

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.97

PROFESSIONAL:

.95

OTHER:

1.02

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Affective characteristics of depressed and well mothers are investigated in relation to the affect and behavior of their children. The moods and emotions observed in the parent and experienced in interaction with the parent are significant socialization influences on the child's own affective development. The affective impairments that are characteristic of depression, if manifested by depressed mothers in their interactions with their children, are likely to create unpredictable, noncontingent, and stressful daily experiences for their children. The questions of interest concern the nature of mother's affective profile, the concordances between mother and child's moods and emotions, the relation of affect to other aspects of child development. When mother and child are analyzed as pairs, there is a high degree of concordance in their affective expression regardless of mother's diagnosis or gender of child. Depressed mother-child dyads are most frequently concordant in negative affect. Mutual positive affect between mother and child is significantly diminished in families characterized by disorganization or chaotic life conditions. The mutual positive affect at 2 to 3 years is predictive of the child's ability three years later to relate competently to an unfamiliar peer.

Project Description:

Assessments of affect were made by continuous coding of mother's and child's affective expressions over three half-days in the laboratory at the time of the first assessment, and again three years later at the follow-up assessment.

Findings:

Not unanticipated is the greater amount of time (number of minutes) coded as negative mood and negative emotions (sad, anxious, irritable, angry) for depressed mothers than for well mothers. However, what is particularly noteworthy is the finding that when mother and child are analyzed as pairs, there is a high degree of concordance in their affective expression regardless of mother's diagnosis or gender of child. When mothers are in the highest quartile on negative affect, 79% of their children are above the median in negative affect; when mothers are in the lowest quartile on negative affect only 15% of the children are above the median. Depressed mother-child dyads are most frequently concordant in negative affect. Mutual positive affect between mother and child is significantly diminished in families characterized by disorganization or chaotic life conditions. The mutual positive affect at 2 to 3 years is predictive of the child's ability three years later to relate competently to an unfamiliar peer.

Previously reported, for part of the present sample, were differences in frequencies of insecure attachment relationships by diagnosis of mother. These findings are replicated in the Fall sample: most insecure attachments in children of bipolar mothers (76%), next most frequent in unipolar mothers (49%), and least frequent in well mothers (36%). When data on mothers' patterns of affect are considered, preliminary findings link high frequency of insecure avoidant attachment with mothers who manifest extreme sadness and anxiety (and sometimes intense affection) in interactions with their children. Children of angry mothers and bipolar mothers are more likely to have anxious resistant attachments. Securely attached children of depressed mothers are being investigated further.

Significance to Biomedical Research:

The offspring of depressed parents are at risk for the development of affective disorders, but the relative combination of genetic and environmental factors is not well researched. This study offers strong evidence of the specific childrearing conditions that may contribute to the development of affective disturbance in young children. The findings are relevant to theories of depression and to issues of prevention.

Proposed Course:

Just beginning are analyses to examine whether affect patterns of the depressed mothers in relating to their children are aberrant in depressive episodes but not between episodes or whether their affect patterns with

their children stem from more enduring personality characteristics of these women and vary relatively little depending on episode status. The sample is such that there are mothers who are in a depressive episode when they are participating in the procedures and mothers who are between episodes. Manuscripts are in preparation.

Publications:

Radke-Yarrow, M., Richters, J., and Wilson, W.E.: Child Development in a Network of Relationships. In Hinde, R. and Stevenson-Hinde, J. (Eds.): Individuals In a Network of Relationships. England, Cambridge University Press, in press.

Radke-Yarrow, M., and Kochanska, G.: Anger in Young Children. In Stein, N.L., Leventhal, B., and Trabasso, T. (Eds.): Psychological and Biological Approaches to Emotion. Hillsdale, N.J., Lawrence Earlbaum Press, in press.

Radke-Yarrow, M., Belmont, B., Nottelmann, E., and Bottomly, L. Young children's self-conceptions: Origins in the natural discourse of depressed and normal mothers and their children. In Cicchetti, D. and Beeghly, M. (Eds.), The Development of the Self During the Preschool Years. New England, Cambridge University Press, in press

Wylie, R.C. Mothers' attributions to their children. In Honess, T.M. and Yardley, K.M. (Eds.), Self and Identity: Individual Change and Development. London, Routledge & Kegan Paul, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02229-03 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Vocalic Analysis of Natural Discourse in Well and Depressed Mothers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. Sherman Research Psychologist LDP/NIMH

Other: Z. Breznitz Assistant Professor University of Haifa
Israel

COOPERATING UNITS (if any)

University of Haifa

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Affective Development

INSTITUTE AND LOCATION

National Institute of Mental Health, NIH, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS:

.72

PROFESSIONAL:

.30

OTHER:

.42

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The speech behavior of 14 depressed and 18 nondepressed mothers during conversation with their 3-year-old children was examined in this study. It was found that (Annual Report #Z01 MH 02229-02 LDP) depressed mothers vocalized less often and responded less quickly to the cessation of their children's speech than healthy mothers. In a mildly stressful situation, (awaiting a doctor's visit), the depressed mothers, but not the healthy mothers, significantly increased their level of speech productivity. Children of the depressed mothers spoke less than children of healthy women, particularly while sitting and eating lunch with their mothers. The observed differences in the mothers' behaviors were interpreted as an indication that the two groups of children are exposed to very different patterns of socialization. The offspring of depressed women are being taught both to keep social interaction to a minimum and to be overreactive to even mild stresses. The differences in the children's behavior may indicate that already these 3-year-old children have learned to keep their interactions with their mother to a minimum. This manner of adaptation may have negative effects on the child's continued social, emotional and cognitive development.

In order to understand the developmental impact of the observed aberrant patterns of vocalic interaction between depressed mothers and their young children, two further studies are underway. First, the question of continuity of experience will be addressed by analyzing the conversation patterns of these women and children three years after the initial observation. Second, the question of alternate environmental supports for the children's development will be examined by assessing the conversation patterns of these young children and their older siblings. These observations are of the children both at the time of the original assessment of mother-child interaction and again at the time of the first follow-up assessment.

Project Description

The purpose of this set of studies is to examine: 1.) the consistency of the patterning of natural discourse of well and depressed women and their young children, and 2.) the alternate environmental supports available to young children from their older siblings.

The procedural details of the study were reported in the original Annual Report in this series (# Z01 MH 02229-01 LDP). Findings presented last year (# Z01 MH 02229-02 LDP) on a sample of 18 normal and 14 depressed mothers and their 3-year-old children were that depressed mothers vocalized less often and responded less quickly to the cessation of their children's speech than healthy mothers. In a mildly stressful situation, (awaiting a doctor's visit), the depressed mothers, but not the healthy mothers, significantly increased their level of speech productivity. Children of the depressed mothers spoke less than children of healthy women, particularly while sitting and eating lunch with their mothers.

The observed differences in the mothers' behaviors were interpreted as an indication that the two groups of children are exposed to very different patterns of socialization. The offspring of depressed women are being taught both to keep social interaction to a minimum and to be overreactive to even mild stresses. The differences in the children's behavior may indicate that already these 3-year-old children have learned to keep their interactions with their mother to a minimum. This manner of adaptation may have negative effects on the child's continued social, emotional and cognitive development.

A question raised by these results concerns the possible long-term sequelae for the children of depressed mothers exposed to this aberrant patterning of communication with their mothers. Two factors are important in projecting long-term effects: one is whether the patterns of interaction seen when the children were 3 years of age are stable. A second is whether the children have alternative sources of social and cognitive support.

Conversation between mother and child, and sibling and child will be examined for 14 (8 girls, 6 boys) children of healthy mothers and fathers, 9 children (4 girls, 5 boys) of major depressed mothers and healthy fathers, and 11 children (4 girls, 7 boys) of major depressed mothers and fathers who also suffer from some form of affective illness. The younger children range in age from 26 to 39 months, the siblings range in age from 5 to 8 years of age. These families are participants in the NIMH Childrearing Study (Annual Report # Z01 MH 02229-01 LDP).

Measures of interest will be total amount of vocalic behavior of each member of the dyad and the time to initiate vocalization after the other member of the dyad has ceased vocalizing.

Significance to Biomedical Research

Dialogue between mother and child is a basic process by which children are socialized and through which children practice aspects of their social and cognitive behavioral systems. This research has demonstrated that fundamental aspects of this dyadic system in depressed mother-child dyads are divergent from that seen in healthy mother-child dyads.

The current study is directed towards identifying if these aberrant patterns of vocalic interaction between young children and their depressed mothers is an enduring aspect of their interaction. If this is found to be true, then it is expected that there will be a long-term cost to the children. One potential source for ameliorating these experiential and socialization differences may be the children's older siblings. Thus, the conversation patterns of the siblings is also examined. Of particular interest is to see if compensatory experiences are provided for children of depressed women through interaction with their siblings.

Proposed Course:

The report on the findings of the original study have been published in Child Development.

Data collection has been completed on the current phase of the project. Data analysis is underway. A report on this second phase of the project will be prepared this year.

Publications:

Breznitz, Z., and Sherman, T.: Speech patterning of natural discourse of well and depressed mothers and their young children. Child Development, 58, 395-400, 1987.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02231-03 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological-Behavioral Relations in Early Adolescence

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.D. Nottelmann	Statistician	LDP/NIMH
Other:	E.J. Susman	Guest Researcher	LDP/NIMH
	G.I. Germain	Research Psychologist	LDP/NIMH
	L.D. Dorn	Guest Researcher	LDP/NIMH
	G.P. Chrousos	Senior Investigator	DEB/NICHHD
	G.B. Cutler, Jr.	Senior Investigator	DEB/NICHHD
	D.L. Loriaux	Chief	DEB/NICHHD

COOPERATING UNITS (if any)

Developmental Endocrinology, NICHD

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.17

PROFESSIONAL:

1.05

OTHER:

.12

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Interrelations of pubertal development and adolescent adjustment and behavior are investigated cross-sectionally and longitudinally. Participants are 9- to 14-year-old boys and girls and their parents. The adolescents and their parents are seen three times, six months apart. Measures of pubertal development include serum hormone levels (gonadotropins, sex steroids, and adrenal androgens), pubertal stage (Tanner criteria), and height and weight. Psychological assessments of the adolescent include adolescent- and parent-report of behavior problems and competencies and behavioral observations of parents and their adolescent in family interactions. Comparisons of ratings of pubertal stage made by medical personnel, the adolescents, and their parents indicate that, with appropriate instruction, adolescents and parents can provide valid assessments of the adolescent's pubertal stage. Analyses involving pre- and postmenarchial girls indicate that mean levels of gonadotropins, sex steroids, and adrenal androgens are higher in postmenarchial than premenarchial girls, but that there is considerable overlap in the distributions. Preliminary analyses suggest that there are similar hormone-behavior relations in pre- and postmenarchial girls. An examination of relations between hormone levels and the adolescents' use of anger and power in family interactions indicates that estradiol and androstenedione levels are related to irritability and assertive forms of aggression shown by girls. For both sexes, there was evidence that controlling behaviors shown by parents and anger shown by adolescents are related in a pattern which results in an escalation of conflict.

Project Description

Interrelations of endocrine, pubertal stage, and physical growth indices of pubertal development and adolescent adjustment and behavior are investigated cross-sectionally and longitudinally across three times of measurement at six-month intervals.

Methods and Findings

The longitudinal sample consists of 54 boys and 49 girls, 9 to 14 years of age, and their parents.

Pubertal development measures include: serum hormone levels, height and weight, and pubertal stage (according to Tanner criteria). Psychological measures include adolescent- and parent-report of the adolescent's behavior problems and competencies, and behavioral observations of parents and their adolescent in family interactions also were made. Details of procedures are given in previous years' reports.

Methodological issues were investigated: Assessments of pubertal stage (Tanner criteria) made by adolescents, parents, and medical personnel were compared. Adolescents and parents rated the adolescent's pubertal stage using photographs of the five stages of puberty. Correlations between adolescent and medical personnel ratings ranged from $r = .77$ to $.91$ ($p < .001$). Correlations between ratings made by parents and by medical personnel ranged from $r = .75$ to $.87$ ($p < .001$). For both adolescents and parents who were not accurate, underestimation occurred in the later stages of puberty; overestimation, in the earlier stages of puberty.

At all three periods of assessment, girls were asked to report on their menarchial status. There were significant discrepancies from one time of assessment to another in their reports of age at menarche and description of their menstrual cycle. For girls who were postmenarchial at Time 1, the length of the menstrual cycle varied from 20 to 54 days. Mean levels of gonadotropins, sex steroids, and adrenal androgens were higher in postmenarchial than premenarchial girls, but there was considerable overlap in the distributions. There are similar hormone-behavior relations in pre- and postmenarchial girls.

Adolescents' use of anger and power in parent-adolescent interactions was examined in relation to hormone status. Although, in general, hormone behavior relations are stronger and more consistent for boys than for girls, in family interactions findings for hormone-aggression relations are stronger for girls. Estradiol and androstenedione levels were related to irritability and assertive forms of aggression shown by girls in the context of family interactions. For boys, findings were sparse, but certain of the significant relations that were obtained -- those between levels of luteinizing hormone, dehydroepiandrosterone, and dehydroepiandrosterone sulphate and degree of aggression -- replicated previous findings for relations between hormone levels and mother-report data of aggressive traits of the adolescent. Aggressiveness in girls appears to emerge only in certain contexts such as family interactions. The family may be

a "safe" place for girls to test power. For both sexes, there was evidence that controlling behaviors by parents and expression of anger by adolescents are related in a pattern that results in escalation of conflict. For both sexes adolescents who reported an absence of psychological support from their parents also reported higher degrees of anxiety and depressive symptoms.

Significance to Biomedical Research

Increases in problems during adolescence are well documented. Findings from this study provide systematic data on behavior change during puberty and begin to clarify how such problems may be related to developmental status and rate of pubertal change in early adolescence.

Proposed Course

Analyses are under way to generate hormone profiles and identify individuals with exceptional profiles of hormone levels. These profiles will be analyzed in relation to physical pubertal change and to competencies and dysfunctions in psychological domains. Ongoing analyses also include predictive analyses; in each case, developmental status and developmental change are used to predict subsequent adjustment and behavior.

Future work includes the examination of mediators in the relations between biological and psychological measures, e.g., social support and parental marital relations and interactions with adolescents.

Publications:

Inoff-Germain, G., Arnold, G. S., Nottelmann, E. D., Susman, E. J., Cutler, G. B., Jr., & Chrousos, G. P. (1987). Relations between hormone levels and observational measures of aggressive behavior of young adolescents in family interactions. Dev. Psychol., in press.

Nottelmann, E. D., Susman, E. J., Blue, J. H., Inoff-Germain, G., Dorn, L. D., Cutler, G. B., Jr., Loriaux, D. L., & Chrousos, G. P.: Gonadal and adrenal correlates of adolescent adjustment. In Lerner, R. M. & Foch, T. L. (Eds.): Biological and Psychosocial Interactions in Early Adolescence: A Life-Span Perspective. Hillsdale, N. J.: Erlbaum, 1987, pp. 303-323.

Nottelmann, E. D., Susman, E. J., Dorn, L. D., Inoff-Germain, G., Loriaux, D. L., Cutler, G. B., Jr., & Chrousos, G. P. Developmental processes in early adolescence: Relations among chronologic age, pubertal stage, height, weight, and serum levels of gonadotropins, sex steroids, and adrenal androgens. J. Adolesc. Health Care. 8: 246-260, 1987.

Nottelmann, E. D., Susman, E. J., Inoff-Germain, G., Cutler, G. B., Jr., Loriaux, D. L., & Chrousos, G. P. Developmental processes in early adolescence: Relations between adolescent adjustment problems and chronologic age, pubertal stage, and puberty-related serum hormone levels. J. Pediatr. 110: 473-480, 1987.

Nottelmann, E. D. Competence and self-esteem during transition from childhood to adolescence. Dev. Psychol. 23: 441-450, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02232-03 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Ability to Concentrate in Children of Depressed and Well Mothers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Z. Breznitz Research Psychologist Univ. of Haifa
Haifa, Israel

Other: S.L. Friedman Health Scientist Administrator HLB, NICHD

COOPERATING UNITS (if any)

University of Haifa, Haifa, Israel
Human Learning and Behavior Branch, NICHD

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Both investigators have taken other positions. Further work on the project will be carried on outside the Laboratory.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02233-02 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Development of Guilt: Language, Emotions, and Behavior

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. Zahn-Waxler	Chief, Sec. on Child Behavior Disorders	LDP/NIMH
Other:	G. Kochanska	Research Psychologist	LDP/NIMH
	S. Denham	Associate Professor	George Mason University

COOPERATING UNITS (if any)

George Mason University

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Child Behavior Disorders

INSTITUTE AND LOCATION

National Institute of Mental Health, NIH, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS:

.92

PROFESSIONAL:

.50

OTHER:

.42

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research focuses on the establishment of methods to investigate the development of guilt in children. Families and children are selected for characteristics (e.g., socioeconomic status, gender, parental depression) that, on theoretical grounds, might be expected to contribute to differential development of guilt and different forms of expression.

Two- to three-year-old children are observed in structured situations in which a problem or mishap occurs. Analyses focus on identification of individual differences in children's reactions. Preliminary results indicate that expressions of guilt and avoidance of responsibility are already present in toddler-age children and take varied functional and dysfunctional forms. Children between 5 and 8 years of age (Z01-MH-02155) are administered a series of pictures of children and adults in circumstances of distress and conflict to tap their propensities for guilt and related affects. In normal control families, children in this age range show the characteristic increases in guilt expected with age, but children with a depressed parent do not. Levels of guilt are lowest in children with parents of low socio-economic status. Guilt scores on this test relate to guilt and anxiety scores in a clinical interview, but only for children with emotionally well parents.

Project Description

Certain mental disorders are characterized by atypical patterns of guilt (e.g., excesses of guilt in depression and deficiencies in sociopathy). Few data are available to indicate why some individuals develop the capacity to maintain healthy, appropriate levels of responsibility, while others experience excesses or deficiencies in guilt at levels that seriously compromise their well-being. This research focuses on the development of methods for investigating patterns of adaptation and maladaptation in the development of guilt in children. Families and children are selected for characteristics (e.g., socioeconomic status, gender, parental depression) that, on theoretical grounds, might be expected to contribute to differential development of guilt and different forms of expression.

Methods Employed and Major Findings

In studies 1 and 2, two- to three-year-old children are observed in structured situations in which a problem or mishap is staged, and the child's tendency to become involved and feel responsible is assessed. In study 1 (N = 70) the focus is on (a) delineation of individual differences in children's patterns of guilt and (b) maternal personality characteristics and mood expressions that may predict differences in children's guilt reactions. Study 2 (N = 65) focuses, in addition, on exploration of mother's and children's verbal communications about emotions with an emphasis on their emotion-induction strategies and their interpretations of causality (e.g., who is responsible and why) in situations of distress.

In study 3, 90 children between the ages of 5 and 8 (Z01-MH-02155) are seen in a semi-structured projective test (a series of pictures of children and adults in circumstances of distress and conflict) which taps their propensities for guilt and related affects and behaviors. Guilt is examined as a function of socioeconomic status, age, gender, and parental depression. In well families, children in this age range show the characteristic increases in guilt expected with age, but children with a depressed caregiver do not. Levels of guilt are lowest in children with parents of low socio-economic status. Guilt scores on the projective test are related to guilt and anxiety scores in a structured clinical interview with these children, but guilt is unrelated to their reports of mood problems. The correspondence between guilt scores on the two measures is found only for children with emotionally well parents.

Boys and girls do not differ on overall measures of guilt. However, in children's interpretations of the distress stories, girls more than boys express concern, distress, and prosocial intentions: themes about the maintenance and termination of interpersonal relationships (separation issues) are most common in girls. High levels of guilt and anxiety are associated with greater concern over relationship issues in girls. In contrast, for boys, the greater the feelings of guilt, the less concern they express about relationship issues. Thus, the dynamics of guilt and its expression are related in complex ways to family background, cultural background, stage of development and sex of child.

Significance to Biomedical Research and the Program of the Institute

Emotions of guilt and related expressions of low self-esteem and worthlessness are major features of psychopathology. Knowledge of the developmental course, the conditions under which different forms of guilt develop, and the circumstances under which guilt begins to become linked with other affective and social maladaptations will contribute an understanding of these aspects of psychopathology. Basic research on processes of development of guilt in children begins to provide this information.

Proposed Course

Data collection has been completed. Data are in various stages of coding, analysis and manuscript preparation. Information derived from these studies will be incorporated into an invited presentation and chapter for the Nebraska Symposium on Motivation. Journal articles are also planned. This project will be completed within the year.

Publications

Chapman, M., Zahn-Waxler, C., Iannotti, R., & Cooperman, G., Empathy and responsibility in the motivation of children's helping. Dev. Psychol., 23(1): 140-145, 1987.

Kuczynski, L., Zahn-Waxler, C., & Radke-Yarrow, M., Development and content of imitation in the second and third years of life: A socialization perspective. Dev. Psychol., 23(2): 276-282, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02234-02 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Infants of Chronically Depressed and Normal Parents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: K. Suter Medical Staff Fellow LDP/NIMH

Other: J. Stilwell Research Nurse Practitioner LDP/NIMH
(Psychiatric)

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Affective Development

INSTITUTE AND LOCATION

National Institute of Mental Health, NIH, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS:

2.02

PROFESSIONAL:

1.00

OTHER:

1.02

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Research has shown that children of depressed parents are at risk for development of affective illness, but little is known about the effects of parental affective psychopathology on infants during the first eighteen months of life. This study examines early affective regulation, attachment behavior, and patterns of mother-infant interaction in infants of two groups of parents: (1) depressed mothers and well fathers, and (2) both parents without psychiatric illness. Parents are screened by structured psychiatric interview (SADS-L) and also complete questionnaire measures of marital adjustment (DAS). Infants and their mothers are observed and videotaped in a set of standard situations in a homelike laboratory setting on two occasions five months apart, beginning when the infants are aged 3 months, 8 months, or 13 months. Data collection has been completed. Data are currently being coded and findings are not yet available. Analyses are planned to examine both individual and group differences in an attempt to look for possible precursors to later difficulties in this at-risk population.

Project Description

Research has demonstrated that children of depressed parents are at risk for development of affective illness themselves; considerable attention has been focused on latency age and adolescent offspring at risk for depression. More recently, this laboratory has examined toddlers and latency age children in families with parental history of affective disorder or no psychiatric diagnosis and has found a number of differences in children and parenting styles between different parental diagnostic groups (see Z01 MH 02144). The purpose of the current project is to look for possible precursors in how early differences are manifest by studying younger infants at risk for later affective illness. Infants of two groups of parents are included: (1) depressed mothers and well fathers, and (2) both parents without psychiatric illness. Attachment behavior, early affective regulation, and patterns of mother-infant interaction are examined.

Parents are screened by structured psychiatric interview (SADS-L). Infants are seen with their mothers in a homelike laboratory setting for two sessions five months apart beginning at one of three ages: 3 months, 8 months, or 13 months. The 13-month group includes 10 depressed mothers and 9 control mothers; the 8-month group, 14 depressed mothers and 9 control mothers; and the 3-month group, 6 mothers in each diagnostic group. Mother-infant interaction is observed and videotaped in a naturalistic setting in a set of standard situations, including introduction to a new environment, free play, introduction of a stranger, brief separation, feeding, and developmental testing. Situations are adapted to be appropriate for each age group. Parents also complete a questionnaire measure of marital adjustment (Dyadic Adjustment Scale) and are interviewed after the second visit about life events and SADS-L status for the interval period. Data are being coded and analyzed for a number of variables, including attachment classification, social referencing, affective regulation, early signs of mastery motivation, and patterns of mother-infant interaction. Comparisons will be made between diagnostic groups as well as examined for developmental and individual differences. Findings are not yet available.

Significance to Biomedical Research

Little is known about the effects of maternal depression on infants. This study will provide data on this issue as well as attempt to identify possible precursors to later difficulties in a population at risk for later affective disorder.

Proposed Course

Data collection has been completed. Data are currently being coded and analyzed. Several manuscripts are planned within the next year.

Publications

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02297-02 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Generosity and Sharing in Children of Normal or Affectively Disturbed Parents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	F.R. Ascione	Guest Researcher	LDP/NIMH
Other:	M. Radke-Yarrow	Chief	LDP/NIMH
	C. Zahn-Waxler	Chief, Sec. on Child Behavior Disorders	LDP/NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Child Behavior Disorders

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.47

PROFESSIONAL:

.05

OTHER:

.42

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The ability to relate positively to other persons is an important aspect of development. One indicator of this achievement is the child's capacity for empathy and ability to help or share with another person. The purposes of this study are to: (1) examine 5-9 year old children's generosity in a laboratory measure of sharing and also in a naturalistic setting with their younger siblings; and (2) explore proximal and distal correlates of generosity and sharing, and their relation to parental diagnostic status (affectively disturbed or normal). (3) There is also a methodological interest in comparing the standard laboratory measure of sharing that has been the basis of the majority of studies of sharing with measures of sharing in the context of the child's family interactions. This study uses data collected during the first phase of a longitudinal study of child rearing and parental affective disorders.

Project Description

The ability to relate positively to other persons is an important aspect of development. One indicator of this achievement is the child's capacity for empathy and ability to help or share with another person. The purposes of this study are to examine 5- to 9-year-old children's generosity in a laboratory measure of sharing and also in a naturalistic setting with their younger siblings and to explore proximal and distal correlates of sharing and their relation to parental diagnostic status (affectively disturbed or normal).

Data for 88 children (60 whose mother and/or father were diagnosed as affectively disturbed) are examined. Mother and two siblings (mean ages 2.5 and 7 years) are observed in an informal apartment setting (Project Z01 HH 02144) and in a variety of contexts over a 2.5 hour period. The interactions are videotaped. The older sibling at one point accompanies the experimenter to another room and participates in a game in which he or she wins candies. The adult model who demonstrates the game donates some of her candy winnings to children who didn't have candy. The older sibling then returns to the apartment and has the opportunity to share the candies he or she has won with the younger sibling and also to share use of a game provided by the experimenter. The sibling pair is alone during the 5 minutes when sharing is assessed.

Levels of and relations among donating, candy sharing, and game sharing are examined. Proximal factors that may be related to generosity and sharing include the frequency and quality of sibling interactions in a period preceding the sharing task and the mother and younger sibling's behavior when the older sibling returns with the candy. Distal factors to be related to generosity and sharing include scores on a "projective" test of the older sibling's affective attributions and aspects of the child's personality (Achenbach's Child Behavior Checklist).

Major Findings

Data from the sharing task have been analyzed with analysis of variance (ANOVA). Results of the ANOVA yielded a significant main effect for age of older sibling. Subsequent analyses indicated that, although the 6 year olds shared significantly less ($M=21\%$) than 5 year olds ($M=36\%$), rates of sharing candy among 5-7 ($M=29\%$), and 8-9 year olds ($M=39\%$) were not statistically different; rates of sharing among 6-7, and 8-9 year olds were not statistically different. No sex differences were found nor was there systematic variation in sharing according to parental diagnostic status. The relations of sharing to child personality variables and sharing to parental diagnostic variables are now being examined.

Significance to Biomedical Research

Empathy and response to other person's needs are important aspects of the development of positive interpersonal interactions. Knowledge of the antecedents and correlates of these behaviors and the possible developmental impairments in children of affectively disturbed parents will provide a basis for understanding the processes contributing to these behaviors.

Proposed Course

Further analytic work is projected to relate the measures of sharing to child personality and familial variables. The work should be accomplished in the next year.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 MH 02361-01 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Relation between Self- and Teacher-reports of Social-emotional Adjustment

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. Sherman Research Psychologist LDP/NIMH
 Other: D. Pellegrini Professor Catholic University

COOPERATING UNITS (if any)

Catholic University

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Affective Development

INSTITUTE AND LOCATION

National Institute of Mental Health, NIH, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS:

.52

PROFESSIONAL:

.10

OTHER:

.42

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One hundred and twenty-three children (the entire group of older siblings in the NIMH Childrearing Study, Annual Report # Z01 MH 0229-01 LDP) 8 - 11 years of age were administered the Child Assessment Schedule (CAS), a structured psychiatric interview. In addition, the children's teachers filled out the Achenbach Child Behavior Checklist - School Version. The Achenbach asks the teachers to report on the children's ability to relate to other children, the children's behavior, and the children's academic achievement.

The question addressed by this study is the extent to which the children's reports of negative affective states, poor social relationships with family members and/or peers, and problems in school correspond to the child's level of adaptation (academic performance, mood regulation, and social relations with peers and adult authority) in the school setting. A secondary question is the extent to which patterns of adaptation in the school context relate to parental psychopathology.

Project Description

One hundred and twenty-three children (the entire group of older siblings in the NIMH Childrearing Study, Annual Report # Z01 MH 02229-01 LDP) 8 - 11 years of age were administered the Child Assessment Schedule (CAS), a structured psychiatric interview. In addition, the children's teachers filled out the Achenbach Child Behavior Checklist - School Version. The Achenbach asks the teachers to report on the children's ability to relate to other children, the children's behavior, and the children's academic achievement.

The question addressed by this study is the extent to which the children's reports on the CAS of negative affective states, poor social relationships with family members and/or peers, and problems in school correspond to the child's level of adaptation (academic performance, mood regulation, and social relations with peers and adult authority) in the school setting.

Significance to Biomedical Research

A fundamental issue in understanding the developmental course of affective illness is to identify significant premorbid signs and/or symptoms. The CAS interview offers the child the opportunity to share with the examiner his/her concerns and feelings about self, peers, and family members. As with adults, children's self reports and internal states are a significant aspect of defining psychopathology. In addition, though, it is important to establish whether these feeling states translate into impairments in life functioning. To our knowledge this form of validation has not been performed on child psychiatric interviews. The contribution of this study will be to indicate the relation for school age children between their symptom picture as revealed in their major work and social setting and their symptom picture as revealed via self report.

Proposed Course

Data collection is underway. The sample will be completed during the following year. When data collection is completed in the spring of 1988, analyses will be initiated.

Publications

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02362-01 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physical/Neurological Development in Children of Healthy and Depressed Mothers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. Sherman

Research Psychologist

LDP/NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Affective Development

INSTITUTE AND LOCATION

National Institute of Mental Health, NIH, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS:

.37

PROFESSIONAL:

.05

OTHER:

.32

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The exciting current work on the biochemical and genetic bases of the major affective disorders makes more salient the need to bridge the sciences and begin to develop concepts and markers linking the physical and neurological substrate with the complex behavioral manifestations of these disorders. A developmental framework provides a basis for moving beyond correlational assessment to causal modeling. This project will be a step in this process.

A rich body of data (Childrearing Study, Annual Report # Z01 MH 02144) on the children's birth complications, physical development, cognitive development and neurological development will be brought together as profiles for individual children. These profiles will be subjected to a cluster analysis. It is expected that some of the clusters of children will be associated with higher family loadings for affective illness. It is also expected that particular clusters will be associated with specific social, emotional, and/or cognitive outcomes, or with the development of symptoms of affective illness, and/or behavioral disorders in the children.

Project Description

The exciting current work on the biochemical and genetic bases of the major affective disorders makes more salient the need to bridge the sciences and begin to develop concepts and markers linking the physical and neurological substrate with the complex behavioral manifestations of these disorders. A developmental framework provides a basis for moving beyond correlational assessment to causal modeling. This project will be a step in this process.

A rich body of data (Childrearing Study, Annual Report # Z01 MH 02144) on the children's birth complications, physical development, cognitive development and neurological development are being brought together as profiles for individual children. These profiles will be subjected to a cluster analysis.

At a correlational level, it is expected that some of the clusters will be associated with higher family loadings for affective illness. At a predictive level of analysis, it is expected that particular clusters will be associated with specific social, emotional, and/or cognitive outcomes in the children. It will be of particular interest to observe if particular clusters are associated with the development of symptoms of affective illness, and/or behavioral disorders in the children.

The specific data sources are:

- 1.) Birth and health records of each child.
- 2.) Height and weight assessments of the younger children (C1) at 2 years of age.
- 3.) Neurological assessments (PANESS Exam) at age 5 for C1, and at age 7-9 for C2.
- 4.) MacCarthy Scales of intelligence for the younger child (C1) at age 5.

Significance to Biomedical Research

The current developments in the genetic and biochemical bases of major affective illness have made more salient the need to address the question of the relation between the identified physiological differences and the complex behavioral symptomatology associated with these disorders. A potential level for beginning to build such a bridge is in the physical and neurological development of individuals who are at risk for the development of affective illness. This study will provide physical and neurological development profiles for children of affectively ill and healthy parents. The children's profiles will be examined to determine if there are groups of children who share developmental profiles.

These developmental characteristics may prove useful for identifying groups of children who share a common physiological substrate for affective disorder. It will be possible, then, to assess the extent to which these children share common social, emotional, cognitive, and/or symptom characteristics as they mature.

Proposed course

The medical records of the children will be collected from family physicians this year. These records will concern birth conditions and child health, and where possible, the age of achievement of the developmental milestones. The PANESS and the McCarthy Scales are being administered. Data analyses will be begun as soon as the data become available, projected in the coming year.

Publications

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02363-01 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Information-Processing Deficits in Schizophrenic Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: T. Sherman

Research Psychologist

LDP/NIMH

Other: R. Asarnow

Associate Professor

UCLA School of
Medicine

COOPERATING UNITS (if any)

UCLA School of Medicine

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Affective Development

INSTITUTE AND LOCATION

National Institute of Mental Health, NIH, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS:

.37

PROFESSIONAL:

.05

OTHER:

.32

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was designed to add to the growing body of literature concerning core cognitive impairments in schizophrenic children. Both adult and child schizophrenics show a wide range of impairments on a variety of cognitive tasks. However, if these studies are examined carefully, it does not appear that we have been able to identify a specific process that is consistently impaired in schizophrenic individuals. This suggests that the impairment is more systemic, not simply contained within a small set of isolable information processing functions. In this study, based on the Feature Integration Theory of Attention by Treisman and Gelade, the ability of the schizophrenic child to recognize the task relevance of, and engage in, serial and parallel modes of visual search is examined. The data reveal that schizophrenic children are as competent as MA-matched control children in their use of a parallel visual search strategy and a serial visual search strategy, and in their recognition of the situations under which each is the optimal strategy. Importantly, the data also reveal that schizophrenic children have a significantly greater start-up time than the MA-matched controls in the initiation of their search strategies. This difference is in the range of 480 msecs. This is much greater than would be predicted on the basis of merely a motor delay in button pushing. These results suggest that the schizophrenic individual does not have a specific information processing deficit, but rather a global deficit in time to initiate the operation of any information processing strategy, be it an automatic strategy or an attention demanding strategy. Such a deficit would be apparent in all tasks requiring speeded information processing, and in real-time processing of information.

Project Description

This project was designed to add to the growing body of literature concerning core cognitive impairments in schizophrenic children. Both adult and child schizophrenics show a wide range of impairments on a variety of cognitive tasks. However, if these studies are examined carefully, it does not appear that we have been able to identify a specific process that is consistently impaired in schizophrenic individuals. This suggests that the impairment is more systemic, not simply contained within a small set of isolable information processing functions. This study, based on the Feature Integration Theory of Attention by Treisman and Gelade, sought to examine the ability of the schizophrenic child to recognize the task relevance of and engage in serial and parallel modes of visual search.

Eight schizophrenic boys and 16 normal boys matched in age and mental age to the schizophrenic children participated in the study. In addition, a developmental study was conducted in order to ascertain whether observed differences between the schizophrenic children and the MA-matched controls could be interpreted as a more immature form of information processing or whether their deficits represent a pattern of information processing not normally seen in developing children. To enable this comparison, there was an adult comparison group (n=9), a comparison group of boys approximately one year older than the schizophrenic children (n=11) and a comparison group of younger boys approximately three years younger than the schizophrenic children (n=11).

Results

The data reveal that schizophrenic children are as competent as MA-matched control children in their use of a parallel visual search strategy and a serial visual search strategy, and in their recognition of the situations under which each is the optimal strategy. Importantly, the data also reveal that schizophrenic children have a significantly greater start-up time than the MA-matched controls in the initiation of their search strategies. This difference is in the range of 480 msec. This is much greater than would be predicted on the basis of merely a motor delay in button pushing and is twice the difference that distinguishes the younger and older normal boys.

These results suggest that the schizophrenic individual does not have a specific information processing deficit, but rather a global deficit in time to initiate the operation of any information processing strategy be it an automatic strategy or an attention demanding strategy. Such a deficit would be apparent in all tasks requiring speeded information processing, and in real-time processing of information. This first suggestion is the result found in the research literature on information processing; the second suggestion is consistent with the clinical picture of schizophrenic individuals.

Significance to Biomedical Research

Research on schizophrenic children provides information on a sample that is less likely than adult samples to have suffered additional impairments due to prolonged drug therapy or institutionalization. In addition, schizophrenic children are probably a more homogeneous group than adult schizophrenics, and

are probably a group with a higher genetic loading for the illness. Thus, research on schizophrenic children offers a potentially purer sample in which to study cognitive impairments associated with schizophrenia. We have found that the information processing deficits in these children are comparable to those seen in adult samples of schizophrenics. This suggests that there is a continuity between adult forms and childhood onset forms of schizophrenia. The current work provides a behavior model of information processing impairment that can guide and inform further research on the brain bases of schizophrenia as well as provide a framework for structured intervention.

Proposed Course

Data collection is completed, and data analysis is underway. A paper is being prepared for presentation in a scientific journal.

Publications

Mundy, P., Sigman, M., Ungerer, J., and Sherman, T. Nonverbal communication and play correlates of language development in autistic children. J. Autism, in press.

Asarnow, R., Sherman, T., and Strandburg, R. The search for the psychobiological substrate of childhood onset schizophrenia. J. Am. Acad. Child. Psychiatry, 26(5), 601-614, 1986.

Mundy, P., Sigman, M., Ungerer, J., and Sherman, T. Defining the social deficits of autism: The contribution of nonverbal communication measures, J. Child. Psychol. Psychiatry 27(5), 657-669, 1986.

Sigman, M., Mundy, P., Sherman, T., and Ungerer, J.: Social interactions of autistic, mentally retarded, and normal children and their caregivers. J. Child. Psychol. Psychiatry 27(5), 647-655, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 MH 02364-01 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Follow-up Investigation of Offspring of Bipolar Parents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	C. Zahn-Waxler	Chief, Sec. on	LDP/NIMH
		Child Behavior Disorders	
Other:	L. Cytryn	Medical Officer (Psych)	LDP/NIMH
	A. Mayfield	Psychology Technician	LDP/NIMH
	D. McKnew, Jr.	Medical Officer (Psych)	LDP/NIMH
	M. Radke-Yarrow	Chief	LDP/NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Child Behavior Disorders

INSTITUTE AND LOCATION

National Institute of Mental Health, NIH, 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS:

.55

PROFESSIONAL:

.25

OTHER:

.30

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Seven male children of severely ill manic-depressive parents (five of whom had a unipolar depressed spouse) were studied at one and at two years of age. They showed a range of adjustment problems when assessed in a laboratory setting (protocol # 78-M-75). They had insecure attachment relationships with their caregivers and had problems in regulation of affect. Disturbances in empathy and aggression also were evident. Psychological and psychiatric assessments of the children were conducted four years later to determine whether problems identified earlier were transitory or persistent and indicated possible precursors of later diagnosable disturbance.

On the follow-up at five and six years of age, these children differed from a control group on many dimensions of functioning. In a psychiatric interview, they reported more fears, worries, symptoms of depression and distortions in self-image. Their mothers indicated a high incidence of externalizing, as well as internalizing symptoms in the children. Proband children received more formal (DSM-III) psychiatric diagnoses than control children. Deficits in empathy and non-assertive strategies for resolving conflict also were identified. Many of the problems persisted over time, even for children who were in therapy, for whom change was evident. These patterns underscore the need for early intervention in families such as these in which both parents are depressed and in which there is a familial history of affective disorder as well.

Project Description

The purpose of this research was to conduct a follow-up evaluation of a small sample of offspring of bipolar parents (annual report # Z01 MH 02155). From a genetic standpoint this is a high risk sample. Five of the seven spouses of the bipolar parents had unipolar depression and all of the seven bipolar parents had a familial history of affective disorder. The environments of their children also differed from those of a control sample: the former were characterized by disorganization, unpredictability, alienation, and weak social support systems. Problems in relationship formation, affective self-regulation, and coping with stress were identified in the proband children when they were infants and toddlers. The main goal of the current research was to determine whether the early problems reflected transitory disturbances, or instead indicated early precursors of later diagnosable disturbance.

Methods Employed and Major Findings

Seven male children with a severely ill manic-depressive parent were seen at ages five and six. Four of the bipolar parents were female and three were males who had been inpatients at NIMH. Diagnoses of bipolar affective disorder were determined on the basis of SADS-L interviews, using RDC criteria. Five of the seven spouses were diagnosed with unipolar depression and one with substance abuse and war neurosis. There was a comparison group of 11 children with nondepressed parents.

At age five the children were observed in a laboratory setting in procedures chosen to assess the areas of difficulty identified earlier in development. The children were observed in interaction with a playmate, under pleasurable and mildly stressful conditions. They were given structured tests developed to assess empathy and conflict resolution strategies. At age six, the children returned for psychiatric and psychological evaluation. A standard psychiatric interview, the Childhood Assessment Schedule, was given. DSM-III diagnoses were determined, based on the child's responses to the psychiatric interview and data obtained from the Achenbach Child Behavior Check List filled out by the mother.

Children in the proband sample reported more fears, worries, and depression than control children on the Childhood Assessment Schedule and they also scored higher on a subset of depression items indicative of suicidal ideation and/or proneness to self-injury. Their mothers reported them to be high on externalizing as well as internalizing symptoms. Psychiatric diagnoses of children indicated that diagnoses were more frequent and of a more serious nature in proband than in control children. Some of the proband children showed continuity over time in the specific symptoms of disturbance while others showed changes in the types of problems. As a group, proband children showed atypical patterns of empathy and social problem solving.

Significance to Biomedical Research and the Program of the Institute

The literature on children with a bipolar parent has yielded mixed findings; few and many problems have been reported in offspring. In some studies these children are described as super competent. It is important to begin to identify

the origins of these differences in order more effectively to plan prevention and intervention strategies. This research provides information about the frequency, nature and severity of symptoms in a group of children for whom there is high genetic loading for affective disorder. The present research helps to provide guidelines regarding some of the specific content areas and problems in need of modification.

Proposed Course

Data collection and analysis have been completed. A manuscript has been prepared and submitted for consideration for publication in the American Journal of Psychiatry. A report based on this work was presented in a symposium on developmental psychopathology at the APA meetings in May.

Publications

Bretherton, I., Fritz, J., Zahn-Waxler, C., & Ridgeway, D. The acquisition and development of emotion language: A functionalist perspective. Child Dev. 57: 529-548, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02365-01 LDP

PERIOD COVERED
October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
The Psychobiological Effects of Sexual Abuse

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F.W. Putnam Staff Psychiatrist LDP NIMH
OTHER: P.K. Trickett Guest Researcher LDP NIMH

COOPERATING UNITS (if any)
Chesapeake Institute, Kensington, Maryland; Montgomery County Child Protection Unit; Prince Georges County Child Protection Unit; Virginia Child Protection Services, Fairfax County, Virginia

LAB/BRANCH
Laboratory of Developmental Psychology

SECTION
Section on Child Behavior Disorders

INSTITUTE AND LOCATION
National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:
.37

PROFESSIONAL:
.25

OTHER:
.12

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study focuses on the psychological and biological effects of sexual abuse on children. Subjects will be sexually abused females (6-15 years of age) and their parents or guardians. Controls will be age and socio-economically matched. The study will use a multi-method approach to gather information on the psychological and physical development of children and their home environment. Methods include: staging physical development, measurement of blood hormone levels, psychological tests and measures for both children and adults. Three hypotheses will be tested: A), that sexually abused girls will have a more difficult transition through puberty; B), that sexual abuse may alter the hormonal levels and effect the timing of puberty; and C), that sexually abused children will demonstrate higher levels of dissociation than controls.

Project Description:

Objectives: Sexual abuse, largely unrecognized until the 1970's, is now known to constitute a major form of child abuse in the United States. While statistics on the incidence of childhood sexual abuse vary widely, even the lowest incidence rates cited indicate that it is a major public health problem. The effects of childhood sexual abuse, both immediate and long-term, are considerable. Prominent symptoms in adults include: depression, suicidality, substance abuse, poor self-esteem, difficulties with concentration, multiple somatic complaints and sexual dysfunction. In children, the data are less clear but frequently reported symptoms include: depression, running away, learning disabilities, self-mutilation, conduct disorders and inappropriate sexual behavior.

This study is the first attempt to follow prospectively a group of sexually abused children longitudinally through puberty. Three hypotheses are tested: 1) that puberty exacerbates the impact of sexual abuse on the psychological development of girls; 2) that certain specific behaviors, i.e. inappropriate sexuality and aggression that are commonly reported in sexually abused children, are associated with elevations in levels of adrenal androgenic and/or gonadotropic hormones; and 3) that dissociative behavior, a psycho-physiological response to extreme trauma, is increased in sexually abused girls compared to matched control subjects.

Method:

The study uses a convergence or cross-sequential prospective design. Psychological development is assessed across two broad domains: 1) indicators of competence and coping with the developmental tasks of puberty and 2) the presence of psychiatric symptoms and behavior problems. Physical development will be assessed using Tanner staging and a series of adrenal and gonadal hormonal measures. Dissociative capacity will be measured using standardized hypnosis scales and the Dissociative Experiences Scale (DES).

Significance to Biomedical Research

This study seeks to document from a developmental perspective the impact of childhood sexual abuse. Three mechanistic hypotheses are tested.

Proposed Course:

This project recently received a large three-year grant from the W.T. Grant Foundation and will begin operation on June 1, 1987. Data will be collected on 50 subjects and 50 control families a year for the first two years. Serial follow-up measurements will begin in year two and be continued on each child for two years.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02366-01 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Psychophysiology of Multiple Personality Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F. Putnam Staff Psychiatrist LDP/NIMH

Other: R. Post Chief BPP/NIMH
D. Weinberger Psychiatrist CBDB/NIMH
T. Zahn Psychiatrist LPP/NIMH
O. Devinsky Psychiatrist LPP/NIMH
N. Hall Psychiatrist George Washington Univ.

COOPERATING UNITS (if any)

George Washington University, Laboratory of Psychology and Psychopathology,
Biological Psychiatry Branch, Clinical Brain Disorder Branch

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Child Behavior Disorders

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.77

PROFESSIONAL:

.65

OTHER:

.12

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study measures reported psychophysiological differences that may exist among the alternate personalities of individuals suffering from multiple personality disorder (MPD). Alternate personalities of subjects with MPD and imaginary personalities of normal simulating control subjects are measured on visual and auditory evoked potentials, spontaneous EEG, cerebral blood flow, continuous catheter venous sampling and measures of autonomic nervous system activity such as galvanic skin response, skin temperature, pulse and respiration.

Project Description:

Objectives: This project seeks to ascertain any psychophysiological differences that may exist among the alternate personalities of individuals suffering from multiple personality disorder (MPD).

Method:

A variety of psychophysiological measures have been used in this study. These include averaged evoked potentials elicited by light and sound in collaboration with Dr. R. Coppola (Neuropsychiatry Branch), galvanic skin response, respiration, pulse and skin temperature (Dr. T. Zahn, Laboratory of Psychology and Psychopathology), cerebral blood flow, (Dr. D. Weinberger, Clinical Brain Disorder Branch), 24-hour continuous telemetry EEG (Dr. O. Devinsky, Laboratory of Psychology and Psychopathology), and circulating immune functions (Dr. N. Hall, Department of Biochemistry, George Washington University).

Findings:

Past findings: Studies from this project indicate that MPD patients can produce changes in visually evoked potentials and spontaneous EEG that cannot be duplicated by simulating control subjects.

New findings: Studies of personality switching, using 24 hour EEG telemetry, indicate that these events are independent of epileptic activity in subjects with concurrent MPD and temporal lobe epilepsy.

Significance to Biomedical Research

The demonstration of state-specific psychopathological responses may permit a more extensive investigation of the role of personality factors in psychosomatic disorders. Multiple personality disorder subjects provide a unique model of the interaction of psychological states and somatic symptoms.

Proposed Course

This study continues to collect data on the psychophysiological responses of alternate personalities in MPD subjects and simulated personalities in control subjects. The most recent focus has been on round-the-clock EEG monitoring of MPD subjects with temporal lobe epilepsy and alternation in the immune functions of MPD subjects.

Publications

Putnam, F.W.: The Scientific Investigation of Multiple Personality. In Quen, J.M. (Ed.): Split Minds Split Brains. New York, New York University Press, 1986, pp 109-125.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02367-01 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Clinical Phenomenology of Multiple Personality Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	F.W. Putnam	Staff Psychiatrist	LDP/NIMH
Other:	R. Lowenstein	Chief	Dept. Psy., UCLA
	R. Post	Chief	BPP/NIMH

COOPERATING UNITS (if any)

UCLA
Biological Psychiatry Branch

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Child Behavior Disorders

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.47

PROFESSIONAL:

.35

OTHER:

.12

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

The clinical syndrome of multiple personality disorder (MPD) is an unusual dissociative disorder that previously had been poorly characterized. Systematic data have been collected on over 200 recent cases of MPD in treatment in the United States. Results indicate that MPD is a clinical syndrome with a core of dissociative and depressive symptoms that occurs in individuals with a history of childhood trauma between ages 2 and 14 years. Male and female MPD patients differ on the expression of aggressive and self-destructive symptoms.

Project Description

Objectives: Until the last decade, multiple personality disorder (MPD) had been considered to be an extremely rare dissociative condition. With the inclusion of MPD in the DSM-III (1980) there has been a dramatic increase in the numbers of reported cases. The purpose of this project is to survey current cases in the United States and other countries to determine the clinical presentation, past psychiatric, medical, childhood and family history of these individuals, the phenomenology of the patient's system of alternate personalities, and the responses of the patient to standard therapeutic interventions.

Method

A 386-item questionnaire, designed to collect detailed information on a single patient, was initially distributed to clinicians reported to be treating MPD patients. A national sampling of patients meeting DSM-III criteria for MPD was obtained and analyzed. Subsequent samples have been obtained on focused samples of MPD patients. Analyses of male MPD patients and MPD patients with concurrent temporal lobe epilepsy are nearing completion.

Findings

Major past findings: The overall patient population was found to be predominately female (92%) with a mean age at sampling of 35.8 years. MPD patients are poly-symptomatic on initial clinical presentation (mean number of symptoms 18.5) with psychiatric symptoms suggestive of depression (depressed mood, mood swings, insomnia, sexual dysfunction and suicidality) and somatic symptoms of headache and GI problems. The average number of alternate personalities was 13.3 with a standard constellation of alternate personality attributes appearing in most patients. A history of childhood trauma was found in 97% of cases.

New findings: Data from this study are being analyzed for variables related to treatment response. In addition, several subsamples of MPD patients have been collected, including males and patients with concurrent temporal lobe epilepsy. Data on male patients indicate that they are more likely to present with a history of recent aggression towards others while females are more likely to be diagnosed after episodes of self-mutilation or suicide attempts.

Significance to Biomedical Research

This project represents the first attempt to conduct a large scale sampling of independent cases of multiple personality disorder. The data from this study are being used to construct profiles of MPD patients to aid in diagnosis and to identify more effective therapeutic interventions.

Publications

Putnam, F.W., Guroff, J.J., Silberman, E.A., Barban, L. and Post, R.M.: The clinical phenomenology of multiple personality disorder: Review of 100 cases. J. Clin. Psychiatry, 47: 285-293, 1986.

Putnam, F.W.: The treatment of multiple personality: State of the art. In Braun, B.G. (Eds.): The treatment of multiple personality disorder. Washington, American Psychiatric Press, pp 175-198, 1986.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02368-01 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Dissociative Experiences Scale (DES)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F.W. Putnam

Staff Psychiatrist

LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Childhood Behavior Disorders

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.37

PROFESSIONAL:

.25

OTHER:

.12

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A short, self-administered questionnaire, the Dissociative Experience Scale (DES), has been developed and tested. The DES reliably and validly measures frequency and types of dissociative phenomena. This instrument has been administered to a wide variety of carefully diagnosed psychiatric groups. The DES has been included in over 50 studies in the United States and abroad and has been translated into French, Dutch and Cambodian. Current research using this instrument includes studies of patients with epilepsy, eating disorders, borderline personality disorder, affective disorders and schizophrenia in conjunction with researchers in the IRP, NIMH; NINCDS; and the Department of Psychiatry, UCLA and the Department of Psychology, The American University.

Project Description

Objective: The phenomenon of dissociation is a complex psychophysiological process that appears to contribute to the psychopathology of a range of disorders, primarily those associated with exposure to traumatic experiences such as posttraumatic stress disorder (PTSD), multiple personality disorder (MPD) and related dissociative reactions. The clinical recognition and the study of dissociative phenomena has been limited by the lack of a reliable and valid instrument to identify and quantify these experiences. A short, self-administered, computer-scored questionnaire was developed that yields an overall index score and three sub-scale scores that reliably and validly quantify dissociative experiences.

Method

Reliability testing has included both test-retest and split-half measures. Validity testing consists of comparing both item and overall scores from a wide range of psychiatric and normal populations. The DES was found to have a significant correlation ($\bar{r} = 0.62$, $\underline{p} = < 0.01$) with another measure of dissociation, hypnotizability, measured by standardized scales. Predictive validity studies are in progress. Our findings for normal adults and adolescents and several psychiatric subgroups have been replicated by other investigators.

The DES was shown to have a significant positive correlation with two standardized scales of hypnotic capacity (DES/SHSS $\bar{r} = 0.62$; $\underline{n} = 60$; $\underline{p} = < 0.01$); DES/HGSHS $\bar{r} = 0.51$; $\underline{n} = 60$; $\underline{p} < 0.01$: Stanford Hypnotic Susceptibility Scale (SHSS) and the Harvard Group Susceptibility to Hypnosis Scale (HGSHS). This provides additional evidence of the validity of the DES to measure dissociative phenomena and indicates that normal individuals with hypnotic capacity frequently have spontaneous dissociative experiences.

The DES was also used with a carefully diagnosed population of Alzheimer's disease patients. It was found that patients with organic memory deficits do not endorse items related to dissociative memory disturbances.

Significance to Biomedical Research

Dissociation is thought to play a major role in the persistence of certain types of symptoms secondary to traumatic experiences, e.g. flashbacks, abreactions, and intrusive thoughts, images or affects. We have developed a reliable and valid means of quantifying dissociative experiences that has been rapidly adopted by researchers working with a variety of psychiatric disorders. While not intended as a diagnostic instrument, the DES has become a widely used screening instrument in clinical settings where trauma patients are common.

Proposed Course

Current DES studies are underway with collaborators from a number of universities and with NIH, to determine the incidence and profiles of experiences of dissociation and depersonalization in temporal lobe epilepsy patients and in eating disorder patients. We plan to employ the DES as a survey instrument on a wide range of carefully diagnosed psychiatric populations. We will continue to increase our samples of schizophrenia, post-traumatic stress disorder, panic anxiety disorder, multiple personality, atypical dissociative disorder, affective, obsessive-compulsive, premenstrual tension syndrome and organic dementia patients. We are also working on an improved form of computer scoring. A pilot study is in progress with the french translation of the DES seeking to explore the issues of cross-cultural expression of dissociative symptoms. Pilot data indicate that the incidence of dissociative phenomena in French normals and psychiatric patients is equivalent to that found in U.S. samples.

Publications

Bernstein, E.M., Putnam, F.W.: Development, reliability and validity of a dissociation scale. J. Nerv. Ment. Dis., 174: 727-735, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02369-01 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mutual Interpersonal Influence in Families with and Without Affective Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Kochanska Research Psychologist LDP/NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.77

PROFESSIONAL:

.35

OTHER:

.42

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Processes of mutual interpersonal influence are studied in dyads comprised of well and depressed mothers and their 5-year-old children. This is a longitudinal study, following up the sample of 87 dyads, in which control and discipline interactions were studied when the children were 1 1/2 to 3 1/2 years old (Z01 MH 02152). Two major themes are followed: difficulties of depressed mothers in controlling their children, which were identified in the original study, and the development of children's competent interpersonal strategies of influence, which in their emerging form were studied in toddler period. In contrast to the first study, where the focus was on the mother as the agent of control and child as a respondent, in this study the focus is on bilateral interpersonal influence processes. Every episode of control, which can be initiated either by the mother or by the child, is coded.

Project Description

Processes of mutual interpersonal influence are studied in dyads comprised of well and depressed mothers and their 5-year-old children. This is a longitudinal study, following up the sample of 87 dyads, in which control and discipline interactions were studied when the children were 1 1/2 to 3 1/2 years old (Z01 MH 02152). Two major themes are followed: difficulties of depressed mothers in controlling their children, which were identified in the original study, and the development of children's competent interpersonal strategies of influence, which in their emerging form were studied in toddler period.

In contrast to the first study, where the focus was on the mother as the agent of control and child as a respondent, in this study the focus is on bilateral interpersonal influence processes. Every episode of control, which can be initiated either by the mother or by the child, is coded.

Method

Ninety minutes of mothers' and children's videotaped interactions in a naturalistic setting are coded. Each episode of interpersonal influence is described regarding its timing, goal, and verbal and physical techniques of influence used by the initiator. Respondent's reaction is described with the use of categories such as compliance and a variety of resistance categories (negotiation, reasoning, refusal, bargaining, excuses, conditional statements, etc.) The interactive qualities of the entire episode are also coded. The coding system is a modified version of the system used in the earlier study, but it reflects children's increased social competence and influence skills. It also allows us to capture the bilateral processes of influence within the dyad, more appropriate at this age. Despite the modifications, the system is compatible with the one used in the earlier study, providing a possibility for longitudinal assessment of the mother-child dyads.

Research Questions

Two major research questions will be addressed: the relation between psychopathology and the processes of interpersonal influence, and the developmental changes between two and five years of child's age. The impairments in control processes of depressed mothers, such as lowered ability to reach compromise with their children and increased tendency to avoid confrontation with the child, were found in the earlier study; the present study will provide not only the opportunity to replicate the findings, but also to study the possible exacerbation of the problems over time, as well as to consider control processes in the context of our much enriched knowledge of psychiatric history of both mother and child. Expectations of developmental change in the processes of interpersonal influence is reflected in our approach to the child as not only a respondent to maternal control, but also as an active agent, initiating control episodes. A rich representation of sophisticated interpersonal strategies, included in the coding system, will allow us to capture further development of social skills, the rudiments of which were described in the earlier study.

Significance of Biomedical Research

The study will provide further insight into the interactive patterns in families with affective disorder. Day-to-day processes of interpersonal influence may be a significant factor in the emergence of disordered patterns of interaction, and therefore may contribute to the increased risk for the development of psychopathology.

Proposed Course

A reliable, modified coding system is in place, and coding has been completed. Analyses are underway.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02370-01 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Caregiving Patterns in Stressed Families

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	F. Bridges-Cline	Guest Researcher	LDP/NIMH
Other:	W.E. Wilson	Research Psychologist	DRG/NIH
	M. Radke-Yarrow	Chief	LDP/NIMH
	D. Hay	Research Psychologist	Univ. of London
	T. Cox	Psychiatrist	Univ. of Liverpool

COOPERATING UNITS (if any)

University of London
Division of Research Grants, NIH
Univ. of Liverpool

LAB/BRANCH

Laboratory of Developmental Psychology
SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.37

PROFESSIONAL:

.05

OTHER:

.32

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Experiences in the family are presumed to play a significant role in the socialization of the child, the development of his or her personality, and the emergence of any pathological characteristics. Of primary interest in this research is the functioning of different sorts of families with respect to a central dimension of family life, the provision of care to its members. An immediate objective of this study is the development of a behavioral family interaction coding system that focuses on the giving and receiving of care among family members. The goal is for the coding system to be at the same time sufficiently broad in the conceptualization of "caregiving" as to be applicable to family life in different cultures and family members of different ages, and sufficiently articulated as to be capable of differentiating families with and without varying kinds of internal and external stressors. An additional aim of the study is the examination of associations of parental depression and other family stressors with varying patterns of family caregiving. This aim derives from the view that family caregiving is a potential mediator of factors such as economic distress or parental pathology that place children at risk for later problems. In this regard, the coding system is being piloted, refined, and used on a sample of disadvantaged families in Britain and a sample of families in the NIMH study, with and without parental depression.

Project Description

The effects of factors that place children at risk for later problems, such as a family's parental psychopathology or economic distress, are likely to be mediated through changes in or dysfunctional patterns of, the provision of care to and by family members. An immediate objective of this study is the development of a family interaction coding system that focuses on the giving and receiving of care among family members. The system is to be sufficiently broad in the conceptualization of "caregiving" as to be applicable to family life in different cultures and family members of different ages, and sufficiently articulated as to be capable of differentiating families with and without varying kinds of internal and external stressors. The unit of analysis that has been selected for this coding system differs from that used in most other observational measures of family process. In contrast to a focus on the individual's action or trait, the emphasis is the events and states in family life to which members of the family may or may not respond, and the family members' perceptions of, and responsiveness to, each other's affective, behavioral, and cognitive states.

In addition, an aim is to examine and describe the varying patterns of caregiving that are associated with variations in family stressors and family background variables. Of primary interest is the factor of parental depression and the way in which this factor becomes manifest in the expression of and response to particular kinds of needs of family members. One question to be asked concerns the extent to which reversals of the usual caregiver role (e.g. an older sibling takes on the role of parent) occur in families with parental depression, and the extent to which it is harmful or beneficial for children to take on caregiving roles in their families.

Method

The major goal of the coding system is to describe how members of a family, parents and children alike, express their needs for care, react to each other's needs, and accept or rebuff whatever care is offered. The starting point for the description of a family's provision of care is the identification of needs that may be perceived or inferred by family members. The observation procedure requires four judgments: 1) determining whether a particular family member is in need of care at a particular time, and characterizing the ways in which the need manifests itself; 2) determining which, if any, of the other family members respond to that need, and characterizing the nature of their response; 3) characterizing the reaction of the person in need to the other family members' responses; and 4) recording instances in which one family member provides or ascertains the need for care for another in the absence of any visible need.

Two studies are involved. One study is the NIMH Childrearing Study; and the other, to be referred to as the London Study, is conducted by Dr. Cox and his colleagues at the University of London. The two studies share some common research goals, as well as diverge in some aspects. Each examines families under stress, due to the affective illness of a parent (NIMH) and conditions associated with economic distress (London study). The issues addressed by

each reflect concern about the extent to which very young children's experiences in the family place them at risk or protect them from the likelihood of later disturbance.

Significance to Biomedical Research

Our goal in this study is to better understand the processes whereby affective illness in a parent may affect the child's experience and understanding of the giving and receiving of care in his/her relationships with others. Clearly, the primary social setting within which the child acquires knowledge and experience of caregiving is the family. The development of a robust observational measure of the family's pattern of caregiving will make available a widely applicable tool for researchers interested in studying significant dimensions of family environment that are associated with differential developmental outcomes.

Proposed Course

The coding system has been developed. Coding of the observational data from U.S. sample has begun, and analyses will proceed as these data are available. A year to 1 1/2 years is an estimated time for this work.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02371-01 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Patterns of Alliance in Families with and Without Parental Depression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F. Bridges-Cline Guest Researcher LDP/NIMH

Other: W. Wilson Research Psychologist DRG/NIH

COOPERATING UNITS (if any)

Division of Research Grants, NIH

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.22

PROFESSIONAL:

.10

OTHER:

.12

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One of the ways in which parental depression may influence the development of the child is through the kinds of affective involvement that characterize the relationships among various members of the family. These patterns of affective involvement within and across family subsystems (i.e. the spouse, parent-child, and sibling subsystems) are significant contributors to the child's development not only through their influence on the pervasive emotional climate of the environment within which the child functions but also through their influence on the child's developing understanding of self in relationships. Within the family systems literature, this aspect of family member relationships (i.e. the direction and degree of affective responsiveness) has been referred to as "alliances"; and it has been found that the pattern and nature of alliances are quite different in distressed and dysfunctional as compared to effectively functioning families. The aim of this study is to examine the nature and pattern of family alliances--the availability and strength of interfamily communication channels and the affective quality of these communications--in families with and without parental depression.

Project Description

One of the ways in which parental depression may influence the development of the child is through the kinds of affective involvement that characterize the relationships among various members of the family. Within the family systems literature, this aspect of family member relationships (i.e. the direction and degree of affective responsiveness) has been referred to as "alliances"; and it has been found that the pattern and nature of alliances are quite different in distressed and dysfunctional as compared to effectively functioning families. For example, distressed families tend to display low overall levels of alliance behavior and a weakness in the marital alliance relative to other family subsystem alliances, for example, the parent-child alliance. The nature and patterning of alliance behavior in families wherein parental depression is the primary stressor has not been examined. Similarly, the role of these differential alliance patterns in child developmental outcomes has not been investigated. The aims of this study are (1) to examine the nature and pattern of family alliances--the availability and strength of interfamily communication channels, and the affective quality of these communications in families with and without parental depression, (2) to identify the nature of alliances within and across family subsystems (spouse, parent-child, and sibling subsystems) and to determine whether there are patterns that distinguish groups of families with and without parental depression, and (3) to determine the association of particular family alliance patterns with differential child developmental outcomes.

Method

The sample is a subset of the Childrearing Study families and includes 45 families who constitute all the families from the larger study in which the two siblings are the oldest two siblings in the family, and in most instances are the only two children in the family. Our reason for selecting these families for the study derives from our concern that we have available from our observations, data relating to all of the immediate family relationship partners that are and have been available at home to the target child(ren) of interest. Patterns of alliance are studied in families with and without parental depression when the two siblings are between the ages of 5-6 years and 8-9 years. Videotapes of the four family members interacting during a mealtime in our research apartment (30 minutes) are used to code interaction patterns. For coding, verbatim transcripts of the conversations of family members are used in conjunction with the behavioral data available from the videotapes. A modified version of the Family Alliances Coding System (FACS) (Gilbert, Saltar, Deskin, Karagozian, Severance, and Christensen, 1981) is used with additional codes developed specifically for this study. The unit of analysis for the FACS coding system is an individual's statement to and/or about another family member. The additions to this coding system that are being developed for this study focus on a more global unit of analysis wherein dialogues or exchanges among several family members on a specific topic serve as the object of a coder's evaluation of communication patterns. The study is not yet at a point where findings are available.

Significance to Biomedical Research

Our goal in this study is to understand better the processes whereby depression in one or both parents may affect the development of the child. A critical place to begin the examination of such processes is at the family system level. Our analytic strategy of examining patterns of alliance that meaningfully distinguish clusters of families allows us to speak directly to the question of the extent to which particular patterns correspond to maternal diagnostic classifications. It is highly likely that such family clusters will cut across diagnostic groups; and to the extent that we see differential outcomes for children in the same family, and children of non-diagnosed as well as diagnosed parents develop problems of a similar nature, this kind of finding would bring us closer to understanding and identifying some of the more significant dimensions of family environment that are associated with differential developmental outcomes.

Proposed Course

All of the 45 verbatim transcripts of the 30 minutes of family conversation during the mealtime have been completed. The coding system and its modifications are in the phase of development and piloting. Plans for the coming year are to complete the coding system development, to complete the coding of the 45 transcripts and to begin analyses.

Publications

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02372-01 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychiatric Status of Children of Depressed Parents

PRINCIPAL INVESTIGATOR (List other personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Radke-Yarrow	Chief	LDP/NIMH
Other:	L. Cytryn	Med. Officer (Psychiatry)	LDP/NIMH
	D. McKnew	Med. Officer (Psychiatry)	LDP/NIMH
	L. Kuczynski	Assoc. Professor	Univ. of Guelph
	T. Sherman	Research Psychologist	LDP/NIMH
	E. Nottelmann	Statistician	LDP/NIMH

COOPERATING UNITS (if any)

University of Guelph, Guelph, Ontario

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.77

PROFESSIONAL:

.95

OTHER:

.82

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

There is very little research that provides prospective data on the developmental course of offspring of depressed parents. At each of the developmental periods at which we have seen the children (younger child at 2 to 3 years, followed up at 5 to 6 years; older sibling 5 to 8 years, followed up at 8 to 11 years), psychiatric assessments have been made, to be related to parental diagnostic status, family environmental variables, and developmental variables. In analyses to date, we have focused on associations between parental diagnoses and children's psychiatric status. At 2 to 3 years of age, assessments did not differentiate the children by diagnosis of parents. About 10% of the children were rated at risk in the well and in the depressed parent groups.

We analyzed the children of middle-class background, who were initially seen as toddlers, at their first follow-up assessment. At this time they manifested problems (DSM-III diagnoses) with different frequencies depending on parental diagnosis, i.e., 15% of the children of well parents, 21% of the children of depressed mothers and well fathers, and 47% of the children of depressed mothers and fathers. When their siblings were seen at 8 to 11 years, the corresponding percentages were 8%, 28%, and 53%. The same data were re-examined in relation to unipolar and bipolar maternal depression. There was little difference in the frequencies of problems in these two groups.

Project Description:

There is very little research that provides prospective data on the developmental course of offspring of depressed parents (Z01 MH 02144). In the present study, assessments are made of children of depressed parents and a comparison group of children of well mothers, at each of the developmental periods at which we have seen the children (younger child at 2 to 3 years, followed up at 5 to 6 years; older sibling 5 to 8 years, followed up at 8 to 11 years). Psychiatric evaluations are part of an assessment battery. Child outcomes are to be related to parental diagnostic status, family history, and family environmental variables. In analyses to date, we have focused on association between parental diagnoses and children's psychiatric status at successive developmental stages

Methods and Major Findings:

Children were evaluated at 2 to 3 years of age in a psychiatric play interview and on a sample of mother-child interactions. Based on these two psychiatric appraisals, children were assigned a score (1 to 4) on degree of presumed risk for later development of psychopathology. These assessments did not differentiate the children by diagnosis of parents: About 10% of the children were rated at risk in the well and in the depressed parent groups. Beginning at 5 to 6 years of age, the children were evaluated by a psychiatric interview, Child Assessment Schedule (CAS), supplemented by mothers' reports on the Achenbach Behavior Checklist. The children who had been seen at the toddler level now manifested problems (DSM-III diagnoses) with different frequencies depending on parental diagnosis. (These analyses are of children of middle-class background.) Problems appeared in 15% of the children of well parents, in 21% of the children of depressed mothers and well fathers, and in 47% of the children of depressed mothers and fathers. In their siblings, who were seen at ages 8 to 11 years, the corresponding percentages were 8%, 28%, and 53%.

The same data were reexamined at ages 5 to 8 in relation to unipolar and bipolar maternal depression. 39% of the children of unipolar mothers and 43% of the children of bipolar mothers manifested problems. In the sample seen at ages 8 to 11 years, 53% of the children of unipolar mothers and 36% of the children of bipolar mothers were given diagnoses.

Proposed Course:

Further detailed analyses are underway in which are specified (a) the kinds of problems in the children, (b) their continuity over development, and (c) their relation to a variety of refinements of parental diagnoses and family variables (specified in project description). A third psychiatric evaluation will be made, beginning this coming year, which brings the children to adolescence. The psychiatric appraisals of the children will be considered along with child outcome data based on observed behavior of child with family and peers, and along with psychophysiological assessments to be made in the follow-up.

Significance to Biomedical Research:

Research on the concordance of psychiatric problems of depressed parents and their offspring is mainly without information on course of development of offspring problems or on the conditions or processes underlying the development of offspring problems or well-being. The data from this study begin to provide such information.

Publications:

Radke-Yarrow, M.: Parental Depression and Parent-Child Interaction. In Patterson, G.R. (Ed.): Family Social Interaction: Content and Methodological Issues in the Study of Aggression and Depression. Hillsdale, New Jersey, Lawrence Erlbaum Associates, Publishers, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02379-01 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Survivor Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Radke-Yarrow Chief LDP NIMH

OTHER: T. Sherman Research Psychologist LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.12

PROFESSIONAL:

.35

OTHER:

.77

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The focus of this study is on children who by criteria of genetic and family variables are at high risk, but who are functioning relatively well. Criteria of high risk were both parents are depressed or mother is depressed and father is absent, and family environment is stressful. Fifty children, two from each of 25 families that met these criteria of risk, were studied. The children were between 5 and 11 years. In-depth case analyses were done on four children who were functioning well; namely, had no diagnosis; were liked by parents, teachers and peers; and were performing at grade level. On this basis, critical factors in their development were identified and hypotheses regarding conditions or processes promoting good functioning were formulated and tested on the 50 children to determine whether the identified factors predicted the current status of the children in the high risk sample.

The protective factors shared by all of the children who were "surviving" were above average intelligence, had socially winning ways or charm, and a match, since birth, between a specific characteristic of the child and a parental need. By fulfilling a specific need of the ill parent(s), the "survivor" children have received the maximum support from their family's scant psychological resources. The children's relatively good functioning is, however, at the expense of their own developmental needs.

Project Description:

The focus of this study is on children who by criteria of genetic and family variables are at high risk, but who are functioning relatively well. This work develops out of a merger of research on risk research and ego resiliency. The objective is to examine the development of children from families in which both parents are depressed or in which mother is depressed and father is absent, and family environment is stressful.

Methods Employed and Major Findings:

Fifty-two children, two from each of 25 families that met these criteria of risk, were studied. The children were between 5 and 11 years. In-depth case analyses were done on 4 children who were functioning well; namely, had no diagnosis, were liked by parents, teachers and peers, and were performing at grade level. On this basis, critical factors in their development were identified and hypotheses regarding conditions or processes promoting good functioning were formulated and tested on the 50 children to determine whether the identified factors predicted the current status of the children in the high risk sample.

The protective factors shared by all of the children who were "surviving" were above average intelligence, socially winning ways or charm, and a match, since birth, between a specific characteristic of the child and a parental need. The group level analyses support the findings on the smaller group. By fulfilling a specific need of the ill parent(s), the "survivor" children have received the maximum support from their family's scant psychological resources. The children's relatively good functioning is, however, at the expense of their own developmental needs.

Proposed Course:

Completed.

Significance to Biomedical Research:

Risk factors in the development of psychopathology have received more attention in research than have factors that are protective. This study focuses on protective conditions. Understanding of processes of developmental psychopathology and of effective intervention depends on clarification of both kinds of factors.

Publications:

Radke Yarrow, M., and Sherman, T.: Hard Growing: Children Who Survive. In Rolf, J., Masten, A., Cicchetti, D., Nuechterlein, K. and Weintraub, S. (Eds.): Risk and Protective Factors in the Development of Psychopathology. Cambridge, England, Cambridge University Press, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02380-01 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Stressful Life Events and Childhood Adjustment

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	J. E. Richters	Research Psychologist	LDP/NIMH
Other:	D. Pellegrini	Psychologist	Catholic Univ.
	M. Radke-Yarrow	Chief	LDP/NIMH

COOPERATING UNITS (if any)

Catholic University

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.77

PROFESSIONAL:

.30

OTHER:

.47

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project is designed to investigate the links between parental psychopathology, stressful life events, and the social-emotional adjustment of children at-risk. The analyses are based on data from families participating in the NIMH Childrearing Project (Z01 MH 02144), which includes children of parents with and without a history of affective disorder. Assessments of parental psychopathology are based on psychiatric (SADS-L) interviews with each parent. Stressful life events to which the children have been exposed are assessed on the basis of intensive, semi-structured interviews with mothers. Evaluations of children's functioning are based on psychiatric interviews, direct observations of their behavior in our laboratory apartment, and independent reports from their parents and teachers.

In an initial set of analyses, characteristics of stressful events (e.g. nature, severity, timing, frequency, saturation) are coded without coders' knowledge of children's responses to the events. Events and characteristics of events that are caused, exacerbated, or buffered by parental functioning are distinguished. Associations between these subsets of stressful events and their characteristics to predictions of offspring adjustment will be examined.

Project Description

The objective of this research is to investigate the links between parental psychopathology, stressful life events, and the social-emotional adjustment of children. A direct influence model holds that stressful life events play a direct causal role in offspring maladjustment by overtaxing the developing child's coping resources and skills. As a result, the child may adopt maladaptive coping responses, and poor self-esteem. An alternative model holds that stressful life events have little or no direct influence on children's adjustment; that it is not stressful events per se, but characteristics of parents that engender and/or exacerbate those events, that give rise to maladjustment and psychopathology in offspring. In this research, data will be examined to relate to these models.

Methods

The analyses are based on data from families participating in the NIMH Childrearing Project (Z01 MH 02144), which includes children of parents with and without a history of affective disorder. Assessments of parental psychopathology are based primarily on psychiatric (SADS-L) interviews with each parent; ratings of maternal and paternal competence/functioning will be derived from direct observation and interview data. Data on stressful life events to which the children have been exposed are obtained in intensive, semi-structured interviews with mothers. Assessments of children's functioning are based on psychiatric interviews, direct observations of their interactions with a same-age peer in our laboratory apartment, and independent reports from their parents and teachers.

Stressful life events are classified by type; direct focus of event; long- and short-term stress-value; control ability timing relative to the child's age; the roles played by each family member in either causing, exacerbating, or lessening the negative impact of each event. Procedures for coding the interviews ensure that the estimated stressfulness of events is not contaminated by the coders' knowledge of the child's functioning.

Proposed Course

To date, 91 mothers and their children have been observed and interviewed in follow-up visits to our laboratory apartment. Life event interviews with mothers have been processed to identify stressful events across these families. A coding system has been developed for the detailed scoring of events, and has been tested on a random sample of event summaries. Coding is in progress, and will be followed by the formal scoring of events. Analyses will then be conducted, and manuscripts will be prepared for publication during this year.

Significance to Biomedical Research

Although the link between stressful life events and psychopathology has been demonstrated repeatedly over the years, the mechanisms through which they are related, and their interrelated impact on children, are not well understood. This research will contribute to our understanding of these links.

Publications

Richters, J.E. Exposure to parental psychopathology and children's adjustment: A developmental analysis. In Hahlweg, K., and Goldstein, M. (Eds.), Family Interaction Research and Psychopathology. New York, Plenum, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02381-01 LDP

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functioning of Depressed Mothers Within and Between Episodes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: J.E. Richters Research Psychologist LDP/NIMH

Other: M. Radke-Yarrow Chief LDP/NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

.77

PROFESSIONAL:

.30

OTHER:

.47

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

What are the characteristic differences between depressed and non-depressed women in their interactions with and around their children? Are maternal child-related behavioral deficits characteristic of women with a history of depression, or are difficulties present only when mothers are experiencing an acute episode of depression? More broadly, are there certain patterns of aberrant child-related behaviors that wax and wane with depressive episodes, and others that reflect more or less enduring behavioral characteristics of depressed mothers? These questions are important to the study of depressed women as mothers and their influence on children.

The NIMH Childrearing Project is in a unique position to explore this issue, by comparing maternal characteristics of two groups of mothers with a history of affective disorder: those who are within and those who are between episodes of depression at the time of their participation in our laboratory apartment at follow-up. These groups of mothers will be compared on symptom-related behaviors and interactive behaviors, including patterns of 1) eliciting compliance and cooperation from their children, 2) monitoring of and responsiveness to their children's need states, 3) displays of affect such as anger and affection, 4) patterns of content and topography in verbal interactions, and 5) methods of resolving conflict between offspring siblings. Parallel analyses will be conducted using self-reported mood as a basis for regrouping mothers; this will allow us to address more general state-trait questions beyond those that are specific to depressive episodes, per se.

Project Description

What are the characteristic differences between depressed and non-depressed women in their interactions with and around their children? Are maternal child-related behavioral deficits characteristic of women with a history of depression, or are difficulties present only when mothers are experiencing an acute episode of depression? More broadly, are there certain patterns of aberrant child-related behaviors that wax and wane with depressive episodes, and others that reflect more or less enduring behavioral characteristics of depressed mothers?

These questions are important to the study of depressed women as mothers and of their influence on children. The NIMH childrearing Project (Z01 MH-02144), is in a unique position to explore this issue, by comparing the maternal characteristics of mothers with no history of affective disorder, with two groups of mothers with a history of affective disorder: those who are in an episode of depression and those who are between episodes of depression at the time of assessments.

Methods

Analyses will be based on data drawn from families participating in the NIMH Childrearing Project (Z01 MH-02144), which includes children of parents with and without a history of affective disorder. Assessments of parental psychopathology are based on psychiatric (SADS-L) interviews with each parent on the day they are observed in the laboratory apartment. To date, 85 mothers and their children have participated in the follow-up phase of this study, including 28 mothers with no history of affective disorder, 33 mothers with a history of affective disorder who were not experiencing a depressive episode at the time of assessment, and 24 mothers with a history of affective disorder who were currently experiencing a depressive episode. Mothers and their children were observed in a variety of situations designed to approximate natural rearing conditions. Mothers will be compared on a selection of symptom-related behaviors, as well as on a range of interactive behaviors that are the focus of related studies on this project, including patterns of 1) eliciting compliance and cooperation from their children (Z01 MH-02132), 2) monitoring of and responsiveness to their children's need states (Z01 MH-02370), 3) displays of affect such as anger and affection (Z01 MH-02207), 4) patterns of content and topography in their verbal interactions (Z01 MH-02220), and 5) methods of resolving conflict between offspring siblings (Z01 MH-02169).

In addition to comparing the previously defined groups of mothers on these characteristics, we will conduct parallel sets of analyses by re-grouping all mothers on the basis of their self-reported (using a standardized battery) moods prior to entering the apartment. These additional analyses will allow us to address more general state-trait questions beyond those that are specific to depressive episodes per se.

Proposed Course

Coding for the maternal behaviors described above is in varying stages of completion. Initial reports may be prepared for publication as early as Spring 1988.

Significance to Biomedical Research

The questions raised and addressed in this research concerning the state-trait dependence of maternal behaviors are of crucial importance to an understanding of the links between maternal depression and offspring adjustment.

Publications

Richters, J.E. and Weintraub, S. Beyond Diathesis: Toward and understanding of high-risk environments. In Rolf, J., Masten, A., Cicchetti, D., Nuechterlein, K., and Weintraub, S., (Eds.), Risk and Protective Factors in the Development of Psychopathology. New York, Cambridge University Press, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00478-31 LN

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neural mechanisms of cognitive memory and habit formation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Mishkin	Chief	LN NIMH
Others:	E.A. Murray	Senior Staff Fellow	LN NIMH
	J. Bachevalier	Visiting Scientist	LN NIMH
	R.C. Saunders	Staff Fellow	LN NIMH
	D.P. Friedman	Project Officer	NRB NIDA

COOPERATING UNITS (if any)

National Institute on Drug Abuse

LAB/BRANCH

Laboratory of Neuropsychology

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

9.0

PROFESSIONAL:

2.5

OTHER:

6.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Every sensory modality in the macaque is served by a series of cortical stations, each of which processes the sensory signal in turn. Signals in the later stations, located in the anterior temporo-insular cortex, can activate a circuit that runs through the limbic system to the modulatory neurochemical systems (e.g. cholinergic, noradrenergic, etc.) and back to the sensory cortical stations. We have proposed that as a result of the action of this circuit on neurochemical release in sensory cortex, some of the neurons whose signals have just represented the sensory stimulus become linked together in a cell assembly that serves as the stored representation of that stimulus. Recognition, say of an object, occurs when an assembly formed on a first presentation of the object is reactivated by its re-presentation on a second occasion. Also, once formed, that assembly can be linked to assemblies representing other stimuli and other events, such as a food reward or a location, thereby investing the recognized object with meaning. The linkage involved in object-reward association appears to be mediated mainly by a limbo-neurochemical circuit running through the amygdala, the medial dorsal thalamic nucleus, orbital frontal cortex, and the basal nucleus of Meynert. Similarly, the linkage involved in object-place association seems to be mediated mainly by a second, parallel limbo-neurochemical circuit running through the hippocampus, the anterior thalamic nuclei, cingulate cortex, and the medial septal and diagonal band nuclei. Each of these circuits has reciprocal connections with one pair or the other of the assemblies described above. Thus, if these circuits have been activated, the sight of the object on a second occasion can lead not only to its recognition but also to recall of both the food reward and the spatial location with which the object had been associated. Recognition and recall are two forms of cognitive memory, both of which can be distinguished from habit formation. The latter form of learning involves stimulus-response association specifically, and we have proposed that such learning depends largely on interactions between the cerebral cortex, and the basal ganglia.

PROJECT DESCRIPTION:

The objective of the studies in this project is to delineate the neural system underlying memory formation in the monkey and to differentiate it from the neural system that underlies habit formation. The methods used include behavioral analyses of the effects of selective cerebral ablations and disconnections combined with anatomical analyses of functional neural pathways. The rationale and design of the studies are often based directly on information derived from other projects in this laboratory, many of which deal with the pathways for, and the mechanisms of, stimulus processing and encoding. The results from these and other projects suggest that the sensory system for each modality is composed of two hierarchically organized corticocortical pathways, one directed ventrally to the temporal lobe limbic system and concerned with object perception, and the other directed dorsally to the frontal lobe motor system and concerned with spatial perception. The ultimate goal of this project is to determine how object and spatial perceptions in the different modalities are formed into memories, how these different memories are associated with each other, how they evoke emotions and motor acts, and how they lead not only to these cognitive events but also to habit formation. Our progress in understanding each of these processes will be described in turn.

(1) Recognition memory

Previous work has indicated that visual recognition memory (assessed by delayed nonmatching-to-sample with trial-unique objects) is mediated by a cortico-limbo-thalamic system composed of two largely separate circuits arranged in parallel. One of these circuits consists of the amygdala, amygdalofugal pathways, and the magnocellular portion of the mediodorsal nucleus (MDmc), and the other consists of the hippocampus, fornix, and anterior nuclear complex of the thalamus (Ant N). The reason for believing that these two circuits operate in parallel is that damage to the amygdalar and hippocampal circuits at each stage in the system (i.e. medial temporal lobe, limbo-thalamic pathways, and medial thalamus) causes a severe loss in recognition memory, but only when the two circuits are damaged in combination. Damage to just one of the two circuits leads to only mild recognition deficits, suggesting that either circuit can compensate for the other as far as recognition is concerned. Recent results indicate that the orbital frontal and anterior cingulate cortical zones, which are related anatomically to the amygdalar and hippocampal circuits, respectively, must also be removed in combination to produce a severe recognition deficit. Removal of either the orbital frontal or anterior cingulate cortex alone, or of prefrontal tissue outside this zone, produces little impairment. Thus, the ventromedial prefrontal region appears to constitute yet another stage in the limbic memory system. In an attempt to determine if the different stages have redundant memory functions, we have begun an experiment that examines the effects on recognition memory of combined ablations of tissue at different stages within a circuit. Preliminary data suggest that combined hippocampal and cingulate cortical ablation leads to more severe memory deficits than ablation of the hippocampus alone. Likewise, combined ablation of the amygdala and orbital frontal cortex leads to a more severe deficit than ablation of the amygdala alone. These new results suggest that the prefrontal

cortical stage of each limbic circuit receives a secondary input from the other circuit.

Recent anatomical evidence has indicated that the bed nucleus of the stria terminalis (BNST) occupies an anatomical position within the amygdalar system that is directly comparable to that occupied by the mamillary bodies within the hippocampal system. That is, just as the hippocampal formation projects to the Ant N both directly and indirectly via the mamillary bodies, the amygdala projects to MDmc both directly and indirectly via the BNST. This anatomical correspondence suggests the possibility that the BNST and mamillary bodies serve analogous functions within the two limbic memory circuits. Since previous work has demonstrated that mamillary body lesions alone produce only a mild recognition memory impairment, we are now testing whether combined damage to the mamillary bodies and BNST will produce a more severe effect. Since fornix transection has been shown to have behavioral effects on recognition memory similar to those of mamillary body lesions, and since this procedure disconnects the mamillary bodies from their hippocampal inputs, monkeys were prepared with BNST lesions plus transection of the fornix. Preliminary results indicate that whereas monkeys with BNST lesions alone or fornix transection alone are only mildly impaired in object recognition memory, monkeys with the combined BNST ablation and transection of the fornix are more severely impaired. These results are consistent with the idea that the BNST participates in recognition memory and that its function in the amygdalo-thalamic circuit could be comparable to that of the mamillary bodies in the hippocampo-thalamic circuit.

Investigations of recognition memory in monkeys have largely been confined to vision. The one exception was an experiment from this laboratory showing that temporal lobe limbic structures are just as important for tactile as for visual recognition. Experiments in another project (MH 02037) have demonstrated that a cortical tactile processing pathway runs from the postcentral somatosensory cortex to the second somatosensory area, SII, from SII to the insular cortex, and, finally, from the insular cortex to the medial temporal region. These results suggest that the insular cortex could play a role in tactile recognition analogous to that played by the inferior temporal cortex (area TE) in visual recognition. We are therefore examining the effects on recognition of resecting the insular cortex. Preliminary results indicate that monkeys with bilateral ablations of this cortex are severely impaired in tactile but not visual recognition of objects. Thus, the results suggest that the somatosensory system, like the visual system, interacts with the limbic system through a series of modality-specific areas. Besides pursuing the tactile recognition studies, we are continuing to explore behavioral methods for evaluating recognition memory in addition so that we can extend our behavioral investigations to this modality.

In addition to examining memory of stimulus quality in each sensory mode, we are interested in studying spatial memory. To this end, we have recently trained monkeys on spatial delayed nonmatching-to-sample in a T-maze, a task that evaluates recency memory for place. The results indicate that monkeys with fornix transections are severely impaired, and that monkeys with cingulate cortical ablations are mildly impaired relative to intact controls. In addition to indicating that the hippocampal system in monkeys is important

for this kind of spatial memory, the experiment helps establish a firm link between primate and rodent memory studies, in that this particular spatial memory task, like most such tasks employed with rodents but unlike most employed with monkeys, involves locomotor responses.

(2) Anatomy of recognition memory

Although we have demonstrated the importance of cortical inputs to the limbic system in object recognition memory, we had not made as much progress in demonstrating the anatomical substrates responsible for the limbic feedback to the cortex that allows memories to be stored. A major hypothesis has been that the basal forebrain cholinergic system plays an important role in this process. But the relations of the basal forebrain to the medial temporal limbic areas and to the midline thalamus had not been completely described. Recent studies have addressed these issues.

Injections of tritiated amino acids were made into the hippocampal formation and amygdaloid complex in order to trace their inputs to the basal forebrain, with the following results. The hippocampal formation projects densely to the medial (Ch1), lateral, and dorsal septum, and to the Ch2 region. There were relatively sparse projections to restricted portions of Ch4 as well. Experiments in which the fornix was transected demonstrated that all of these projections ran through the fornix. The caudal hippocampus projects to the more medial septum, whereas the rostral hippocampus projects to the more lateral septum, although there is considerable overlap in the projections from these two regions. The regions of the basal forebrain and septum receiving hippocampal inputs project back to the hippocampus.

The projections from the amygdala do not overlap those from the hippocampus, but terminate instead in the Ch3 and Ch4 fields of the substantia innominata. In fact, the amygdala represents one of the major inputs to Ch4, which provides the major portion of the cholinergic input to the cerebral cortex. The amygdalar efferents arise from the medial, medial basal, magnocellular portion of the accessory basal, and the central nuclei. It is the more dorsal and medial portions of this group that receive the largest number of intrinsic amygdalar connections. The lateral nucleus, which is the site of termination of afferents from the major sensory systems, provides almost no outputs to the basal forebrain.

Because lesions of the medial thalamus lead to deficits in recognition memory like those caused by medial temporal damage, and because the medial and midline thalamic nuclei receive inputs from the hippocampal formation and amygdaloid complex, the structures occupying this region appear to be intimately involved in memory formation. To examine medial thalamic outputs, we initially made large injections of tritiated amino acids that filled the entire rostrocaudal and dorsoventral extent of the thalamic midline. Subsequently we made smaller isotope injections into some of the individual nuclei along the midline, including nucleus reuniens (Re), the magnocellular division of the medial dorsal nucleus (Mdmc), and the nuclei of the anterior group. Because of the expected sparse nature of these projections, autoradiograms were exposed for up to a year before they were developed and analyzed.

The large injections revealed an extensive cortical projection system that ran in long bands along the outer half of layer I and commonly crossed the borders between cortical fields. These projections were seen in ventrolateral, orbital, and subcallosal regions of prefrontal cortex, virtually the entire extent of the temporal lobe ventral to the superior temporal gyrus, the visual fields of the occipital and parietal lobes, the entire extent of the insula, much of the frontoparietal operculum including gustatory and somatic fields, and the auditory areas that border the ventral insula. In addition, cortical limbic areas, including the cingulate gyrus, entorhinal and perirhinal areas, the subicular fields, and the hippocampus proper, were labeled. In addition, there were dense patches of label in the Ch4 fields of the basal forebrain.

Injections into MDmc led to dense label in layer IV of orbital cortex and some patches of label in the overlying layer I as well. The injections into Re produced only layer I label in subcallosal and orbital cortex. Projections originating in the anterior nuclei and the medially adjacent nuclei of the midline led to label in both layers I and III in the subcallosal region.

The findings supply evidence for a widespread system of midline thalamic projections to layer I of higher-order sensory and limbic cortex. These layer I projections could well form part of a cortico-limbo-thalamo-cortical feedback circuit involved in the formation of new memories.

(3) Associative memory

Our earlier work suggested that although the amygdalar and hippocampal systems contribute equally to recognition memory, they have selective roles in associative memory. The amygdala, but not the hippocampus, appears to be important for the association of object qualities from different sensory modalities. In contrast, the hippocampus, but not the amygdala, appears to be important for the association of object quality and place. To test these generalizations, monkeys are being trained on a visual-visual associative memory task. Preliminary results indicate that, compared to hippocampectomized monkeys, those with amygdalar ablations are retarded in relearning a preoperatively acquired set of visual-visual associations. Interestingly, however, neither operated group appears to be impaired in learning new sets. The critical question now is whether monkeys with combined ablations of the amygdala and hippocampus will be impaired in learning new sets. If so, it would suggest that monkeys with limbic lesions resemble amnesic humans, who cannot learn new arbitrary associations no matter how much training they receive. Alternatively, if monkeys with the combined ablation can learn new sets, it would show that some nonlimbic mechanism can contribute to intramodal stimulus-stimulus learning, a mechanism that might be related to the priming observed in amnesic humans.

We have also begun to examine the role of the hippocampal system in conditional spatial learning, where which object of two is positive depends on their spatial location. In one such task, monkeys were required to use the location of the objects within the room as the conditional cue guiding their choice, whereas in another they were required to use the location of the objects relative to themselves to guide their choice. In both tasks, the monkeys were moved passively to the test site, thus eliminating "active

movement through space" as a possible cue. Interestingly, monkeys with fornix transection were not impaired on either task, and monkeys with ablations of cingulate cortex were mildly and transiently impaired on the first but not the second. These experiments lay the groundwork for an analysis of spatial memory in normal monkeys as well as the role of limbic structures in such memory.

(4) Habit Formation

Whereas monkeys with limbic lesions generally exhibit poor memory on recognition and associative memory tasks they are able to learn certain types of object discriminations at a normal rate. For example, we have found that such monkeys can learn as rapidly as normal control animals to discriminate 20 pairs of objects presented concurrently, even with intertrial intervals lasting 24 hours. We have applied the label "habit formation" to this and other examples of preserved learning ability following limbic-system lesions. Normal monkeys trained on the 24-hr concurrent discrimination test with several successive sets of objects learn the later ones faster than the earlier ones. To investigate the basis of this phenomenon, we are examining whether it is affected by limbic lesions. The results show that monkeys with combined amygdaloid and hippocampal ablations, like normal monkeys, learn the later sets faster than the earlier ones, suggesting that the phenomenon of learning set formation is supported by nonlimbic mechanisms.

In a separate LN project (MH 02039), we have been attempting to examine the role of the neostriatum in 24-hr concurrent learning by disrupting the entire nigro-striatal dopaminergic system with the selective neurotoxin MPTP. Because preliminary results with two different regimens of MPTP administration suggest that there is little effect, if any, at doses that do not also produce motor impairments, we are planning to test the effects of damaging selectively those portions of the neostriatum to which the cortical visual system projects (see LN project MH 02033).

Another behavioral paradigm that may provide a measure of the ability to acquire habits is delayed nonmatching-to-sample (DNMS). At very short delays, monkeys with limbic lesions can relearn the delayed nonmatching rule to a high level of accuracy. It is unlikely that the same temporo-neostriatal pathway presumed to be responsible for simple visual discrimination habits could mediate DNMS, however, because in DNMS the monkey must avoid the previously baited sample in favor of a novel object (i.e. it must learn a counter-reinforcement win-shift rule). One nonlimbic circuit that might mediate such complex rule learning is a temporo-prefronto-neostriatal circuit, which could act both to suppress the win-stay rule that is normally mediated automatically by the direct temporo-neostriatal pathway and to mediate in its place the acquisition of the higher-order win-shift rule. To test this possibility, we are investigating whether the combination of inferior prefrontal and limbic ablations interferes with performance on DNMS even at very short delays. If animals with such lesions were unable to relearn the nonmatching rule, it would open up a new chapter in frontal lobe research, namely, the role of the prefrontal cortex in the formation of complex habits.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

In the process of investigating the role of various temporal lobe structures in the visual memory of monkeys, we obtained a result that is particularly exciting because it appears to solve the long-standing puzzle concerning the neuropathology underlying the syndrome of global anterograde amnesia in man. This syndrome, which is characterized by a profound inability to remember new experiences or acquire new information, has been attributed in the clinical literature to destruction of the hippocampus. Yet, attempts to duplicate this syndrome in monkeys by removal of the hippocampus alone have largely failed. What we have found in our studies is that if damage to the hippocampus is combined with damage to the amygdala then a profound memory loss does ensue. The discovery has not only resolved the discrepancy between clinical and experimental findings in nonhuman primates, but has also provided new insight into the neural substrates of memory. Specifically, it has led to the development of a hierarchical model of recognition and associative memory involving a cortico-limbo-neurochemical loop that may well serve as the foundation for all cognitive processes beyond perception, including thought. As we gain further understanding of the memory system, and how it differs from the noncognitive system for habit formation, we will inevitably gain a better understanding of thought and its breakdown in normal and abnormal behavior.

PROPOSED COURSE OF RESEARCH:

Having found severe object recognition losses in both vision and touch after lesions of the limbic system, we shall continue our attempts to devise tests of auditory recognition and visual spatial recognition, with the aim of determining whether the limbic system is indeed critical for recognition in all perceptual modalities. Also, further attempts will be made to differentiate between amygdalar and hippocampal contributions to associative memory, and we shall test whether any functional distinctions that apply to these temporal lobe structures are carried further through the thalamic, prefrontal, and neurochemical segments of the two limbic circuits. In addition, we shall continue our exploration of the neural basis of habit formation, with particular attention initially to the neostriatal and prefrontal targets of the occipitotemporal visual system.

PUBLICATIONS:

Aggleton, J.P. A description of the amygdalo-hippocampal interconnections in the macaque monkey. Exp. Brain Res. 64: 515-526, 1986.

Aggleton, J.P., Friedman, D.P., and Mishkin, M. A comparison between the connections of the amygdala and hippocampus, with the basal forebrain in the macaque. Exp. Brain Res. (in press)

Mishkin, M. and Appenzeller, T. The anatomy of memory. Sci. Amer. 255: 80-89, 1987.

Mishkin, M. and Phillips, R.R. A cortico-limbic memory path revealed through its disconnection. In C. Trevarthen (Ed.): Brain Circuits and Functions of the Mind: Festschrift for Roger Wilcott Sperry. Cambridge University Press, New York. (in press)

- Murray, E.A. and Mishkin, M. Experimental studies of memory in monkeys: implications for understanding human memory disorders. National Forum, Spring 1987, p. 33-37.
- Nelson, R.B., Friedman, D.P., O'Neill, J.B., Mishkin, M., and Routtenberg, A. Gradients of protein kinase C substrate phosphorylation in primate visual system peak in visual memory storage areas. Brain Res. (in press)
- Phillips, R.R., Malamut, B.L., Bachevalier, J., and Mishkin, M. Dissociation of the effects of inferior temporal and limbic lesions on object discrimination learning with 24-hour intertrial intervals. Behav. Brain Res. (in press)
- Saunders, R.C. and Rosene, D.L. A comparison of the efferent connections of the amygdala and the hippocampus. I. Convergence in the entorhinal, prorhinal and perirhinal cortex. J. Comp. Neurol. (in press)
- Saunders, R.C., Rosene, D.L., and Van Hoesen, G.W. A comparison of the efferent connections of the amygdala and the hippocampus. II. Reciprocal and non-reciprocal connections. J. Comp. Neurol. (in press)

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02032-11 LN

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neural coding of visual stimuli in the awake monkey

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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Others: L. Optican Senior Staff Fellow LSR NEI
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INSTITUTE AND LOCATION

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TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Single neurons were recorded from the lateral geniculate nucleus and primary visual cortex, the first two extraretinal stages of visual processing, and inferior temporal cortex, the last visual cortical processing station, to study the mechanisms underlying visual perception. Neurons in all three regions showed different temporal response patterns to different visual stimulus patterns. When these neurons were analyzed as communication channels carrying information about visual stimuli in their responses, the response patterns seen could only be represented as the sum of several (3-6) simultaneous, independent patterns of activity. Three of these activity patterns were analyzed as a temporal code, and the information contained in them was compared to the information conveyed by the number of action potentials, the usual measure of neuronal response; there was twice as much information available in the temporal code. This showed that each response can be considered the sum of activity from several independent channels, with each channel acting as a spatial-to-temporal filter. Traditionally, it has been thought that information about multiple stimulus parameters, such as luminance, pattern, and duration of presentation, must be confounded in the neuronal responses. However, based on this multiplex-filter hypothesis, a new analysis of the primary visual cortex responses led to the discovery that information about each of these parameters is carried separately in the response. This strongly suggests that a consistent neural code exists. A geometrical analysis of these data shows a potential structure for this code. When a 3-dimensional space is used to represent the responses, the responses to a single pattern appear to lie in a plane regardless of luminance or duration. For a single neuron's response, the planes for different patterns are frequently separable. The equations for these planes describe a neural code.

PROJECT DESCRIPTION:

Objectives:

The ability to perceive, recognize, and discriminate visual patterns requires the cooperative function of a sequentially connected system of cortical brain regions extending from primary visual cortex through inferior temporal cortex. The functionality of these regions arises from the properties of the single neurons in them. Thus, to understand how visual perception occurs, we must learn how information is encoded by the neurons in these successive stages of processing. One clear consequence of such understanding would be the ability both to predict the single neuronal signals to arbitrarily constructed stimuli, and to decode the responses to unknown stimuli. We have been recording single neurons in several regions of the visual pathway with the goal of developing a quantitative model of neuronal function. Such a model should simulate the activity of single visual system neurons in response to any arbitrary stimulus conditions. The development of such a model would confirm our understanding of the functionality of these neurons, and, therefore, would be a major step toward understanding the functional role of single neurons in the networks that underlie higher visual function such as perception and memory.

Major findings:

We have developed an unbiased approach to identify and quantify the message-carrying features of single neuronal spike trains. In this approach single neurons are considered to be communication channels that convey messages about visual stimuli. Adoption of this approach has allowed us to identify and quantify the stimulus related information carried by single neurons through the use of techniques from multivariate statistics, Shannon's information theory, and signal processing.

To apply this approach, both the inputs (visual stimuli in this case) and the outputs (neuronal responses) had to be precisely described. For inputs, we constructed a set of 2-dimensional black and white stimuli from a set of basic signal elements (Walsh functions) that can be used to represent any picture. The responses were described in terms of an optimal (in the mean squared error sense) set of statistically derived, independent patterns of activity, the principal components. This set of 2-dimensional black and white stimuli was then used to stimulate neurons in both inferior temporal and primary visual cortex. For single neurons in both regions, a statistical analysis we developed showed that three or more principal components were related to the stimuli presented. Application of Shannon's information theory showed that the amount of information transmitted about the stimulus was twice as great when three principal components were used to represent the responses as when the spike count was used to represent the responses. Thus, the stimulus-related responses of these neurons are multidimensional, i.e., several parameters are required to describe them.

From this, we inferred that each neuron can be viewed as a small (3) number of simultaneously active spatial-to-temporal filters. In this multiplex-filter hypothesis, each filter gives rise to a temporally modulated message that

corresponds to a different aspect of the visual pattern. The output of the filters, represented by the principal components, are then multiplexed onto the spike train. This multidimensional description is a significant advance over less rich, unidimensional descriptions of neuronal responses. Neither static receptive field models nor response strength measures can correctly predict the temporally modulated responses of a neuron. However, a model based on this multiplex-filter hypothesis has predicted the temporally modulated responses of striate cortex complex cells to arbitrarily constructed black and white patterns. Thus, knowledge of the multidimensional nature of the responses allowed derivation of a predictive model of stimulus-related neuronal responses.

Two issues that arise as a consequence of this hypothesis have been investigated during the past year. First, can the responses be decoded? To do this a neural code and its meaning must be identified. Our strategy has been to study the responses of neurons with stimuli that varied along several dimensions. Second, what do the spatial parts of the spatial-to-temporal filters look like?

The experiments that led to the multiplex-filter hypothesis were carried out using stimuli for which the pattern varied while other parameters remained unchanged. However, the strength of a neuron's response is modulated by many different stimulus dimensions, e.g., color, shape, and luminance. According to a common view of neuronal function, the strength of a neuron's response represents how closely the stimulus matches the receptive field's characteristics, e.g., orientation or color. Thus, if response strength were the only parameter a neuron could use to encode information, different stimulus features would be confounded by individual neurons.

Under the assumption that information is carried in a single parameter, the response strength, information about multiple features can be unconfounded only by looking at the distribution of activity over a large population of neurons, although no explicit solution to this problem has been shown. In light of our finding that stimulus features modulate not only the number but also the temporal distribution of spikes in a neuron's response, it is possible that information about multiple features may not be confounded if the multivariate nature of the response is taken into account.

To study whether the encoding of information about different stimulus features might be separably encoded in neuronal responses, single neurons were recorded from primary visual cortex of awake, trained rhesus monkeys. While the monkeys fixated, a complete set of 16 one-dimensional, optimally-oriented Walsh patterns were flashed on the receptive fields of the neurons one at a time. Each pattern was presented at each of four luminance combinations (luminance range: 0.004 - 5.0 ft-lamberts), and flashed for each of five durations (32 - 256 ms). All 320 stimulus combinations were presented to eight cells. The principal components were extracted from 320 ms segments of the densities, and the information transmitted about each of the different stimulus parameters was calculated.

Transmitted information measures the discriminability among inputs given an output for a pair of input and output codes. Information is low when inputs

are confounded among outputs. We related four stimulus input codes to each of two response output codes. The input codes were: 1) the four luminances alone, 2) the five durations alone, 3) the 16 patterns alone, and 4) the 320 combinations of these features. The two output codes were: 1) the coefficients of the first three principal components (T3) and 2) the number of spikes in the response (Ts).

	all 320	pattern	duration	luminance
T3(bits)	1.07 \pm .08(SE)	0.32 \pm .04	0.20 \pm .05	0.19 \pm .02
Ts(bits)	0.46 \pm .04	0.15 \pm .02	0.06 \pm .02	0.05 \pm .01

The information encoded by three principal components, T3, was two to four times greater than the information encoded in the spike count, Ts, for all input codes. Ts for pattern was much greater than that for duration or luminance, whereas T3 had approximately the same magnitude for all three features. Strikingly, the transmitted information based on the input code of all 320 stimulus combinations (1.07 bits) was substantially greater than that predicted by the sum of the information from the individual codes (0.71 bits). Thus, the codes for the three stimulus features were synergistic: more was known about the stimulus when a code that simultaneously accounted for all three features was used.

Similar synergy was also observed in another seven cells tested with 16 patterns and 7 luminances at one duration. This synergism implies that the different stimulus features were not confounded by the neuron. For such synergy to occur, there must be a consistent, unconfounded, mapping from stimulus features to neuronal responses. However, information theory does not require that the neural code be interpretable in terms of these features.

To search for a description of the encoding rules, i.e., a neural code, the responses of the 15 striate cortical neurons described above were analyzed further. The principal components used to quantify the responses are orthogonal patterns of temporal activity that define the axes of a multidimensional space. Responses with different temporal waveforms have different amounts of each principal component in them, and, therefore, they map to different points in this principal component space.

In a plane defined by two principal components, no relation between stimulus features and neuronal responses was apparent. However, in a space defined by the first three principal components, the responses elicited by an individual Walsh pattern, irrespective of duration or luminance, appeared to lie near a plane. Thus, we approximated the responses to each of the 16 patterns by their best-fit planes. The average residual variance per plane was 4.6% \pm 4.1 (SD, n = 112) for the 7 neurons tested with 7 luminances, and 12.0% \pm 5.2 (n = 128) for the 8 neurons tested with 4 luminances and 5 durations. Since the coefficients of the principal components are uncorrelated, if the responses to a given pattern had not been well approximated by a plane, the residual variance would have been close to 33%.

In any one neuron's principal component space, many of the planes representing the 16 stimulus patterns appeared easily differentiable. This geometrical structure demonstrates that the generation of neuronal responses obeys certain rules, which form an intrinsic temporal neural code for visual features. A response could be decoded to determine the stimulus pattern irrespective of duration or luminance if the plane into which the response falls could be ascertained. Information about duration and luminance would then be encoded relative to that plane. Since three points determine a plane, such a decoding scheme may require as few as three complementary neurons sharing related codes.

In another set of recently begun experiments, data have been collected to determine the spatial part of the spatial-to-temporal filters for lateral geniculate nucleus neurons. The first station of the visual system outside of the retina is being investigated for this project because it has been extensively studied and its properties from conventional experiments are well characterized. These neurons have been shown over the past 30 years to have receptive field properties that have excitatory centers with inhibitory surrounds. Our analysis reveals that, even in this early stage of visual processing, information is carried by more than one temporally modulated message. Thus, there appears to be previously unsuspected richness of information in the temporal modulation of the responses. From these data, collected in collaboration with members of the NEI's Laboratory of Sensorimotor Research, we have developed a technique to estimate the spatial distribution of the filters that give rise to each of the temporally modulated messages. These estimates seen thus far suggest that the classical center-surround mechanism may arise from the combined influences of two filters. The structures of the estimated filters suggest that these neurons may encode a nonparametric estimate of the visual scene, possibly its luminance profile, and the first derivative of the luminance profile. If this can be confirmed, it would suggest that lateral geniculate neurons simultaneously encode information about the luminance and luminance gradient of the scenes that fall within their receptive fields.

Our results imply a new functional role for neurons in the visual system. Information about stimulus features is conveyed by individual neurons through multiple messages carried by a temporally modulated code. Consolidation of local messages to determine global properties of images may be accomplished through compilation of many temporally encoded messages. Processing of information in visual areas may consist not so much in altering the distribution of active elements as in transforming temporally modulated messages. We suggest that the hierarchical organization of feature abstraction supported for the multiple visual areas is better replaced by a process of successive stages of spatial-to-temporal filtering that change the emphasis of the visual features but never confound or ignore information.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Disorders of attention, perception, and memory accompany many psychiatric and neurological disorders. This project studies how information is encoded and transmitted. The knowledge gained will ultimately aid in the design of strategies both for more effective palliative treatment of cognitive deficits and for restitution of cognitive function.

PROPOSED COURSE OF RESEARCH:

Discovering that the responses of visual system neurons are multidimensional suggests a completely new conceptual framework in which to investigate neuronal function. This led to the discovery that information about multiple stimulus features may not be confounded by single neurons, a result with important, even revolutionary consequences. One presumed reason for the huge number of single neurons has been the necessity to unconfound stimulus features. If this need not be done on the global scale previously envisioned, what are all these neurons doing? This issue is critical.

Since we have evidence that a neural code may exist, and we have seen a possible structure for it, we will pursue its delineation. The properties of the code should give clues about the functions performed by the neurons. Also, the structures of the spatial filters seen in the lateral geniculate nucleus have already given some new ideas about the properties of encoded information there. Both these issues are being pursued.

New experiments are planned to make multiple, simultaneous single neuronal recordings. The responses from these single neurons will be related to each other through use of recent extensions to methods of signal identification. With these methods to extend our techniques, it should be possible relatively rapidly to develop models that describe the roles of single neurons as components of larger networks. These studies should yield a better understanding of the information transmission mechanisms used for cognitive functions such as pattern discrimination and recognition.

PUBLICATIONS:

Optican, L.M. and Richmond, B.J. Temporal encoding of two-dimensional patterns by single units in primate visual cortex: III. Information theoretic analysis. J. Neurophysiol. 57: 162-178, 1987.

Richmond, B.J., Optican, L.M., Podell, M., and Spitzer, H. Temporal encoding of two-dimensional patterns by single units in primate visual cortex: I. Response characteristics. J. Neurophysiol. 57: 132-146, 1987.

Richmond, B.J. and Optican, L.M. Temporal encoding of two-dimensional patterns by single units in primate visual cortex: II. Quantification of response waveform. J. Neurophysiol. 57: 147-161, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02033-10 LN

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional mapping of sensory and memory systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	R.C. Saunders	Staff Fellow	LN NIMH
Others:	M. Mishkin	Chief	LN NIMH
	J. Bachevalier	Visiting Scientist	LN NIMH
	C. Kennedy	Guest Researcher	LCM NIMH
	L. Sokoloff	Chief	LCM NIMH

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Laboratory of Cerebral Metabolism, NIMH

LAB/BRANCH

Laboratory of Neuropsychology

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The cerebral areas related to vision in the rhesus monkey were identified by comparison of metabolic activity in visually stimulated versus visually deafferented cerebral hemispheres. The results allowed delineation of the visual-nonvisual borders of both an occipitotemporal and an occipitoparietal visual pathway and specification of their points of interaction with frontal, limbic, striatal, and diencephalic structures. In addition, it was found that, within the occipitotemporal pathway, the forebrain commissures contribute to the visual activation of area TE only.

PROJECT DESCRIPTION:

Metabolic mapping of the primate visual system

The extent of the two main cortical pathways known to be critical for higher order visual functions, the occipitotemporal and occipitoparietal pathways, were revealed in a comprehensive picture of the entire visual system at work, achieved by application of the [^{14}C] 2-deoxyglucose method. The method was applied while monkeys, restrained in a primate chair, either 1) passively viewed a high-contrast geometric pattern mounted on a surrounding rotating drum, or 2) actively performed a visual pattern discrimination task that required a response with the hand opposite the deafferented hemisphere. Earlier, the monkeys had received either an optic tract section combined with forebrain commissurotomy, and thus had one hemisphere visually deafferented, or had an optic tract section only, and thus had one hemisphere only partially deafferented. The comparison of local cerebral glucose utilization (LCGU) in the visually deafferented versus intact hemispheres within the same animal made it possible to identify and delineate the areas related to vision, whereas comparison of LCGU in the totally versus partially deafferented hemispheres across animals allowed assessment of the contribution to vision made by the forebrain commissures.

The 2DG metabolic mapping procedure yielded the following picture. All cortical tissue caudal to the junction of the lunate and the intraparietal sulci is related to vision. Nonvisual tissue first appears in the superior parietal lobule (probably somatosensory) and at the beginning of the lateral fissure (probably auditory). In the parietal lobe, the upper border always remains within the intraparietal sulcus, about halfway down the upper bank caudally and closer to the fundus rostrally. The lower border moves from the lateral fissure and into the intraparietal sulcus rostrally. The rostral limit of visual tissue is located within the intraparietal sulcus, about 5mm behind its anterior tip. In the temporal lobe, the upper border always remains within the superior temporal sulcus, generally about halfway down the dorsal bank caudally but within the fundus rostrally. The lower border moves from the calcarine fissure to the hippocampal sulcus (where it continues midway along its length) and then turns laterally to enter the occipitotemporal sulcus and finally the fundus of the rhinal sulcus.

Subcortically in the temporal lobe, tissue related to vision occupies the lateral and lateral basal nuclei of the amygdala, posteroventral putamen, ventral claustrum, and the tail of the caudate nucleus. Visual tissue is also present in the anterior part of the head of the caudate nucleus, known to receive input from the visually related cortex of the inferior frontal convexity, and in both the body and the posterior portion of the head of the caudate nucleus, known to receive input from the visually related posterior parietal and prearcuate frontal cortices.

Comparisons of LCGU along the occipitotemporal pathway in the hemispheres with total and partial visual deafferentation revealed a significant difference only in area TE, reflecting a contribution of the forebrain commissures to visual activation of this region alone. The likely explanation for the failure of commissural fibers to activate glucose metabolism in any part of

the occipitotemporal pathway posterior to area TE is that the primary function of the commissural input to the posterior part of the pathway is to provide suppressive rather than excitatory influences on neural activity (see LN project MH 02036).

Metabolic mapping of the visual memory system

The two conditions of visual stimulation used for metabolic mapping of the visual system in the perceptual studies described above yielded virtually identical results. We are now attempting to map the further processing of visual information by requiring monkeys to perform a visual learning task. In addition, instead of using the single-label 2-DG paradigm employed in the perceptual studies, we will use the recently developed double-label technique, which will allow us to map brain metabolism related to two different types of learning in the same subject. The double-label 2-DG procedure entails separate, sequential injections of ^{14}C -2DG and ^3H -2DG in an individual subject, with each injection immediately followed by a different experimental condition. Thus, the metabolic activity in the brain accompanying each experimental condition is indexed separately by the two radioactive labels. To enable mapping of the cognitive memory system, monkeys will be trained to perform the visual memory task, delayed nonmatching-to-sample (DNMS). This test has been used extensively to assess the effects of cortical, limbic, and thalamic lesions on memory (see LN Project MH 00478). For the present investigation the task has been modified so that the monkey's memory will be stimulated continuously throughout the experimental session. To enable mapping of the visual habit system for comparison, the same monkeys will also be trained on a visual discrimination task. Performance on this task has been shown to be unaffected by the same limbic lesions that disable the cognitive memory system. After reaching a performance criterion of 90% correct responses in both tasks the monkeys will be prepared with a forebrain commissurotomy combined with a unilateral amygdalectomy plus hippocampectomy. The monkeys will be retrained so that they perform at high levels even on the most difficult stages of the memory task. The double-label 2-DG method will then be applied with the animals performing the discrimination task after the first label is injected and then the memory task after the second label is injected. We expect to be able to map the processing of visual information in both hemispheres beyond the areas seen in the visual perceptual studies. In the intact hemisphere (without the limbic system ablations) we expect to be able to visualize the proposed limbo-thalamic memory system, and in the hemisphere with the memory system shut down, we expect to be able to visualize structures related to the 'habit' system.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

The 2-deoxyglucose method provides a unique method of relating neural structure and function, permitting for the first time both the visualization and quantification of local levels of metabolic activity simultaneously throughout the entire brain in animals studied either under normal conditions or following experimental intervention. The results continue to provide important insights into the role of various cerebral structures, both cortical and subcortical, in particular behaviors. Our initial studies contributed valuable information as to the tissue occupied by the two cortical visual

processing pathways, and we expect equally valuable information from our investigation of the two cortico-subcortical learning systems. The new double-labelling methods should prove to be an especially powerful tool for this purpose.

PROPOSED COURSE OF RESEARCH:

Besides pursuing the metabolic mapping of learning processes, we expect to apply the double-labelling 2-deoxyglucose method to the study of a variety of behavioral processes in the monkey, including not only perception and memory but also attention, emotion, and volition, for the purpose of identifying the various structures involved in these different behaviors and quantifying the degree of their participation.

NOTICE OF INTRAMURAL RESEARCH PROJECT

201 MH 02035-07 LN

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Anatomy of the primate visual system

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)

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Others:	M. Mishkin	Chief	LN NIMH
	R. Desimone	Research Psychologist	LN NIMH
	D. Boussaoud	Visiting Fellow	LN NIMH
	R.J. Tusa	Assistant Professor	Johns Hopkins Univ.

COOPERATING UNITS (# any)

Johns Hopkins University

LAB/BRANCH

Laboratory of Neuropsychology

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

4.75

PROFESSIONAL:

2.0

OTHER:

2.75

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type Do not exceed the space provided.)

To better understand the role of visual association cortex in perception and memory, we have been examining the functional areas that comprise this cortex in the macaque and exploring their interconnections by the use of neuroanatomical tracing techniques in combination with electrophysiological recording of neural activity. Our results indicate that a multiplicity of separate visual areas lie beyond the primary visual cortex, or striate cortex (V1), in the stream of information processing. These areas are organized into two divergent corticocortical pathways, each having V1 as the source of its initial input. One, an occipitotemporal pathway, enables the visual recognition of objects, while the other, an occipitoparietal pathway, mediates the appreciation of the spatial relationships among objects as well as the visual guidance of movements toward objects in space. The visual areas along the occipitotemporal pathway (V1, V2, V3, V4, and areas TEO and TE of the inferior temporal cortex) appear to be organized primarily as a serial hierarchy, in which each area processes both color and form information. By contrast, the areas along the occipitoparietal pathway (V1, MT, and MT's projection zones in parietal cortex) process the direction of stimulus motion. Because a major component of this pathway extends anteriorly within the superior temporal sulcus, the neural mechanisms underlying visuospatial function may be more extensive than previously thought. To establish the links of both the occipitotemporal and occipitoparietal pathways with the motor system, we have been exploring the projections of visual association cortex to the striatum. So far, we have found that TE, TEO and V4 all project to the tail of the caudate nucleus and ventral putamen, an arrangement that contrasts with the pattern of projections from the occipitotemporal pathway to the limbic system, which arise from TE only. The presence of direct projections to the striatum but not to the limbic system from V4 and TEO may explain the ability of monkeys with TE lesions to acquire visual habits but not visual memories.

PROJECT DESCRIPTION:

The long-term objective of this project is to understand the role of visual association cortex in perception and memory. To this end, we have been examining the multiple functional areas that comprise this cortex in the macaque and exploring the complex circuitry of their interconnections. So far, we have discovered that the primary visual area, striate cortex, is the source of two divergent corticocortical pathways: one, an occipitotemporal pathway, which enables the visual recognition of objects; the other, an occipitoparietal pathway, which mediates the appreciation of the spatial relationships among objects as well as the visual guidance of movements towards objects in space. A major question to be answered in the future is how the object and spatial information carried in these two separate pathways are subsequently integrated anatomically to yield a unified visual percept.

METHODS EMPLOYED:

In these studies, we have used a variety of neuroanatomical tracing techniques (e.g., amino-acid autoradiography, horseradish peroxidase histochemistry, axonal transport of fluorescent dyes) in combination with electrophysiological recording of neural activity in anesthetized monkeys. In addition, we have employed histological stains that clearly distinguish, for the first time, differences in architecture among the multiple visual areas.

MAJOR FINDINGS:

1. An occipitotemporal pathway for object vision

We had previously found that the occipitotemporal pathway begins with the projection from the striate cortex, or V1, to the second and third visual areas, V2 and V3, which project in turn to area V4. These three prestriate areas are arranged in adjacent cortical belts that nearly surround the striate cortex, and, like the striate cortex, each belt contains a representation of the contralateral visual field. The major output of V4 is to a widespread region within the inferior temporal cortex. Within posterior inferior temporal cortex, or architectonic area TEO, label was found only after V4 injections involving the representation of the central 10° of the visual field, whereas within anterior inferior temporal cortex, or architectonic area TE, label was found after injections of any part of V4. Thus, central but not peripheral field representations in V4 project to TEO, while both central and peripheral field representations in V4 project to TE.

Physiological studies have shown that TE has no discernible visuotopic organization. Rather, neurons in TE have very large receptive fields that nearly always include the center of gaze and frequently cross the vertical meridian into the ipsilateral visual field. Thus, a single neuron in TE can "see" an object no matter where it occurs in the field, which is in keeping with the role this area plays in object recognition. Surprisingly, nothing is known about the properties of neurons within TEO. As a first step in studying these properties, we have begun to map TEO electrophysiologically. Thus far, we have found that the receptive fields of neurons in TEO are mainly foveal, which is consistent with the input this area receives from the central field

representation of V4. It remains to be seen whether TEO is visuotopically organized, like the prestriate areas that precede it, or whether it is nontopographic, like area TE. Because lesions of area TEO, unlike those of area TE, produce impairments in pattern perception rather than in object recognition, a physiological comparison of TEO with TE should help in understanding the neural mechanisms of these functions.

2. An occipitoparietal pathway for spatial vision

We had previously found that although area MT receives inputs from areas that participate in the occipitotemporal pathway (i.e. V1, V2, V3, and V4), its outputs appear to be mainly to areas located in the parietal lobe. One projection zone, area VIP, lies ventrally in the anterior two-thirds of the intraparietal sulcus, while two others, areas MST and FST, are located on the dorsal bank and floor, respectively, of the superior temporal sulcus. To examine the role these areas play in visual function, we have recorded the electrophysiological properties of neurons within MT's projection zones and compared these properties with those of neurons in MT itself. Our results indicate that, like neurons in MT, a majority of those in MST and a third in FST are directionally selective. Compared to neurons in MT, however, neurons in both MST and FST integrate motion information over progressively larger portions of the visual field and respond selectively to more complex types of visual motion. To determine additional components of this motion-analysis system, we have injected multiple anterograde and retrograde tracers into physiologically identified locations within MST and FST of five monkeys.

The major connections of MST and FST are with each other and with areas in the superior temporal sulcus (STS) and the posterior parietal cortex. In the STS, both MST and FST project to cortex on the dorsal bank of the sulcus including portions of the superior temporal polysensory area (STP), which contains many cells with complex directional selectivity. MST has particularly heavy connections with a region at the bottom of the dorsal bank, which may either be part of STP or a new visual area. In contrast, FST is connected with an adjacent area in the floor of the sulcus. In the posterior parietal cortex, both MST and FST have connections with area V3A in the parieto-occipital sulcus, and with cortex in the intraparietal sulcus, including but not limited to areas VIP and LIP; these areas are known to project in turn to the inferior parietal lobule. Finally, MST also has connections with medial parietal cortex, including but not limited to area PO and the cingulate gyrus.

The results suggest that the cortical pathway for motion analysis, which begins with the projection of V1 to MT, splits into at least two components. One component includes widespread regions of the posterior parietal cortex, whereas the other extends into the temporal lobe and includes several areas on the dorsal bank and floor of the STS. Thus, the neural mechanisms underlying visuospatial function may be far more extensive than previously thought. While it is known that lesions along the parietal component of this system cause impairment in spatial perception, eye movements, and visually guided hand movements, the effects of lesions along the temporal component of this system remain to be explored.

3. Projections of visual association cortex to the striatum

We have recently begun to explore the projections of visual association cortex to the striatum. Neurobehavioral studies in our laboratory suggest that these projections are the ones that mediate visual habits, whereas projections from visual cortex to the limbic system mediate visual memories. We are particularly interested in determining the visual cortical areas that project to the striatum, how the projection fields relate to one another, how the cells of origin are organized, and to which structures the visual portions of the striatum project. Our long-term goal in this study is to establish the links of both the occipitotemporal and occipitoparietal pathways with the motor system.

So far, we have placed multiple anterograde and retrograde tracers into the tail and genu of the caudate nucleus or adjacent ventral putamen under physiological control in five monkeys. After caudate injections, labeled cells were found both in a large continuous region of cortex topographically related to the site of injection, and in several non-continuous cortical regions. After injections in the rostral tail, the continuously labeled region included rostral area TE and adjacent portions of TF, TH, TG, and, occasionally, area 35. After injections into the posterior tail and ventral genu, the labeled region shifted posteriorly to posterior TE, TF, and TEO and then into prestriate areas V4, MT, PO, and (sparsely) V3 and V2. As the injection site advanced into the dorsal genu and then to the caudal body, the labeled region shifted toward the parietal lobe, to area 7, to areas VIP and LIP in the intraparietal sulcus, and into area 5 and adjacent area 23. The non-continuous areas labeled by nearly all injections included the principal sulcus/frontal eye field region, area 24, the superior temporal polysensory area, and, more rarely, area 25. Thus, whereas certain temporal, occipital, and parietal cortical areas project into the striatum largely according to proximity, prefrontal, anterior cingulate, and superior temporal sulcal areas have a wider distribution. In all cases, labeled cortical cells were found mostly in layer 5 but also in layer 3.

The finding that areas TE, TEO, and V4 all project to the tail of the caudate nucleus and ventral putamen contrasts with the pattern of projections from the occipitotemporal pathway to the limbic system, which arise from TE only. The presence of direct projections to the striatum but not to the limbic system from areas V4 and TEO may explain the ability of monkeys with area TE lesions to acquire visual habits but not visual memories.

Anterograde labeling following injections into the tail of the caudate nucleus or ventral putamen was confined to the globus pallidus (GP) and substantia nigra pars reticulata (SNr). Because of the known projections from the GP and SNr via the thalamus to the supplementary motor and dorsolateral prefrontal cortex, respectively, these cortical regions may represent further stations in the neural circuit underlying the formation of visual habits. Ultimately, we hope to delineate the entire wiring diagram of this circuit.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

An understanding of the basic mechanisms mediating normal visual perception and memory is the first step in the diagnosis, alleviation, and, ultimately, prevention of sensory, perceptual, and mnemonic disorders. To this end, we have been exploring projections out of the striate cortex to prestriate association areas. Our goal has been to trace the complex system of projections stepwise to the still higher-order visual areas located within the temporal and parietal lobes, areas critical for object vision and spatial vision, respectively. The combined use of axonal transport techniques and electrophysiological recording provides a powerful tool for tracing neural connections within these central visual pathways. In addition, the recent development of highly selective histological stains may give us the opportunity for the first time of identifying the same higher-order visual areas in the human brain that we have identified in the monkey.

PROPOSED COURSE OF RESEARCH:

Thus far, we have found that visual cortex in the monkey is organized into two divergent corticocortical pathways and that the projections of both pathways can be traced from the striate cortex through multiple prestriate association areas to the still higher-order visual areas in the temporal and parietal lobes. Our recent studies suggest that both the temporal and parietal lobes also consist of multiple visual areas, and we will continue to investigate their organization. Since there are no direct connections between temporal and parietal cortex, a major question to be answered is how the object and spatial information carried in these two separate pathways are subsequently integrated anatomically. We also plan to continue our investigations of the links of both pathways to affective memory, and motor systems by examining the projections of the multiple visual association areas to limbic structures, the neostriatum, and prefrontal cortex. Finally, using a combination of histological stains and degeneration techniques on human brain material with occipital lobe lesions, we will attempt to identify the multiple visual areas in the human cortex that have been differentiated in the monkey.

PUBLICATIONS:

Segraves, M.A., Goldberg, M.E., Deng, S.-Y., Bruce, C.J., Ungerleider, L.G., and Mishkin, M. The role of striate cortex in the generation of eye movements in monkeys. J. Neurosci. (in press)

Tusa, R.J. and Ungerleider, L.G. Fiber pathways of cortical areas mediating smooth pursuit eye movements in monkeys. Annals Neurol. (in press)

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02036-07 LN

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neural representations of visual stimuli in the extrastriate cortex

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TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

1.25

OTHER:

1.25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

Disorders of perception, attention, and memory frequently accompany the major mental diseases. To begin to understand the neural mechanisms of these mental processes, we are recording the activity of neurons in the extrastriate cortex of monkeys engaged in tasks requiring visual discrimination, selective attention, and recognition memory. We have found in area V4 of the occipital cortex and area TE of the inferior temporal cortex that selective attention gates visual processing by filtering unwanted information from the receptive fields. Even the degree to which attended stimuli are processed in these areas depends on "how much" attention or effort is devoted to them. Thus, the information-processing capacity of cortical neurons depends not only on hard-wired mechanisms but on cognitive state. To identify the mechanisms by which cognitive state modulates cortical activity, we have begun to examine both extrastriate neuronal activity and animal behavior in an attention-demanding task following lesions of selective portions of the prefrontal cortex, posterior parietal cortex, limbic-system, and basal ganglia.

PROJECT DESCRIPTION:

This is a long-term project to understand both the neuronal basis of perception and memory in extrastriate cortex and the mechanisms by which these processes are influenced by cognitive factors such as selective attention. We focused our initial work on the basic sensory information coded by neurons in the extrastriate areas most directly involved in object recognition. Having identified several of the dimensions along which extrastriate neurons code objects, we have now turned to examining the dynamic operation of this cortical system in awake monkeys engaged in tasks requiring selective attention and memory.

Methods employed: Our most recent studies were carried out in awake monkeys, trained to hold their gaze steady while they performed a match-to-sample task. In this task, the monkey held a bar while a stimulus appeared briefly at one retinal location followed shortly by a second, briefly presented stimulus at the same location. The monkey was rewarded for releasing the bar immediately if the two stimuli matched and for releasing the bar after a fixed delay if they did not match. At a second retinal location, two other, irrelevant stimuli were also presented on each trial, each concurrently with one of the stimuli used in the task. After a block of trials at the first location, a cue was given, and the previously irrelevant stimuli at the second location became the relevant (and, therefore, attended) stimuli for the matching task. Thus, identical sensory conditions were maintained across trials, but the locus of the animal's attention varied.

Experiments:

1. Neural mechanisms for the analysis of form and color in area V4. Anatomical experiments in our laboratory have shown that visual area V4 is a central station in the pathway from the primary visual cortex to the object recognition system of the temporal cortex. We have completed an extensive analysis of sensory coding in V4 and have identified for the first time many of the different stimulus features used by V4 cells to code objects, including the color, length, width, orientation, and contrast of contours, and the spatial frequency, phase, and overall size of sinusoidal gratings. One full-length paper on this work has been published and two more are in preparation. Now that we have a better understanding of sensory coding in V4, we will concentrate our future studies on the mechanism by which coding is controlled by attention.

2. Selective attention gates visual processing in extrastriate cortex. Earlier in this project, we found that when a monkey attends to a stimulus within the receptive field of a neuron in either area V4 or area TE of extrastriate cortex, the processing of other, distracting, stimuli within the field is blocked. Thus, unwanted stimuli appear to be filtered out of the visual system as a result of selective attention, explaining why we perceive and remember only a small portion of the stimuli acting on our retinas at any given time. In subsequent experiments, we have found that extrastriate neuronal responses to a given stimulus are larger and more tightly tuned when the monkey is discriminating that stimulus from one that is very similar to it than when the monkey is discriminating it from one that is very different.

These results suggest that even the degree to which attended stimuli are processed depends on "how much" attention or effort is devoted to them. A short report on these findings has been published and full-length reports are in preparation. Having established that information processing in extrastriate cortex is controlled by cognitive state, our next step is to understand the neuronal basis of this control.

3. The source of the attention "gate". We found previously that the neuronal effects of spatially directed attention do not occur in either the primary visual cortex or area V2, so that whatever structure or structures gate extrastriate responses to attended stimuli must work at the level of V4 and beyond. Anatomical studies in our laboratory have identified at least four possible direct sources of modulating inputs to V4 and/or inferior temporal cortex, namely portions of the pulvinar, posterior parietal cortex, prefrontal cortex, and limbic system. In addition, recent studies of the effects of unilateral dopamine depletion by Dr. Doris Doudet in LCM suggest that dopamine in the substantia nigra may play an indirect but very important role in attentional modulation of cortical activity. To determine the relative role of these structures in the control of attention, we will test individually the effects of reversible deactivation of the lateral pulvinar and portions of the limbic system (using the GABA agonist muscimol), unilateral lesions of the dopaminergic system in the substantia nigra (using the neurotoxin MPTP), and removal of specific areas of the parietal and frontal cortex on the ability of monkeys to perform our attention task with distracting stimuli. As a control for the possibility that any lesion effects we observe are actually due to impairments on the non-attentional components of our task, we will also measure the monkeys' performance on a version of the task that does not contain distracting stimuli and is, therefore, not attention-demanding. A group of animals has now been prepared for the study and are about to undergo behavioral testing.

4. Neural mechanisms for recognition and associative memory. Although monkeys and man exhibit agnosia following damage to the cortex of the temporal lobe, there is remarkably little neurophysiological evidence that memories are actually stored in the cortex. To test this possibility, we have developed a system for presenting many complex visual and auditory stimuli in recognition and associative memory tasks. Our initial recordings from neurons in area TE of the temporal lobe in monkeys performing a recognition task have been encouraging, in that they indicate that many cells do indeed respond differently to a visual stimulus depending on whether or not it has been presented previously to the monkey. Since we have so far only tested for these differences over very short time intervals, we do not yet know if these responses could form the basis for a long-term recognition memory. This possibility will be examined over the next year.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

The results from our recording experiments in extrastriate cortex demonstrate that psychological factors such as selective attention and effort directly affect the cortical neurons that process incoming stimuli and store items in memory. If, during the future course of this project, we can determine exactly how these effects take place, we will be in a better position to understand,

and, ultimately, treat the disorders of perception, attention, and memory that frequently characterize major mental diseases such as Alzheimer's disease, Parkinson's disease, and schizophrenia.

PROPOSED COURSE OF RESEARCH:

A major thrust of our work over the next year will be to continue to track down the structure or structures that modulate cortical processing as a result of selective attention. Our general strategy will be to first identify likely sources of the modulating input by testing the effects of lesions on the attentional capacities of monkeys. When a structure has been so identified, we will then record from its neurons while the animal performs our selective attention task, looking for neuronal responses related to the animal's switch of attention. Finally, we will attempt to model the system with computer-simulated neuronal networks.

PUBLICATIONS:

Albright, T.D. and Desimone, R. Local precision of visuotopic organization in the middle temporal area (MT) of the macaque. Exp. Brain Res. 65: 582-592, 1987.

Desimone, R. and Schein, S.J. Visual properties of neurons in area V4 of the macaque: Sensitivity to stimulus form. J. Neurophysiol. 57: 835-868, 1987.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02037-06 LN

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional anatomy of the somatosensory cortex of the monkey

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TOTAL MAN-YEARS:

0.25

PROFESSIONAL:

0

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

To identify a route by which tactile information could reach limbic structures in the temporal lobe, we used axonal transport techniques to trace the connections between somatosensory cortical fields. On the basis of the laminar patterns of these corticocortical connections, we identified them as 'forward' or 'backward' by analogy to similar designations in the visual system, where they have been shown to have functional validity. The analysis indicated that a forward-projecting route could be traced from the subdivisions of the primary somatosensory cortex to the second somatosensory area, SII; from SII to the granular and dysgranular fields of the insula; and from the insula directly to the amygdala and indirectly to the hippocampus via rhinal cortex. This multisynaptic cortico-limbic pathway in the somatosensory system is thus organized in a manner analogous to the multisynaptic cortico-limbic pathway in the visual system. To assess the functional importance of the pathway, we studied the somatosensory receptive fields of neurons in SII cortex following selective ablations within the primary somatosensory cortex and found that elimination of any given representation of the body surface in the postcentral strip eliminated it also in SII. For example, removing the hand representation from the postcentral strip resulted in its disappearance from SII cortex; conversely, removing all other body representations from the post central strip (i.e. except that of the hand) resulted in the preservation in SII of the hand representation only. The electrophysiological data thus provide strong support for the conclusion, based originally on the anatomical data, that tactile information is processed sequentially along a corticocortical pathway. The electrophysiological experiments also revealed a surprising degree of functional reorganization in SII cortex following the postcentral cortical ablations. After each partial removal, the vacated representation in SII was filled in by the expansion of the intact, neighboring representations. These last findings point to a previously unrecognized degree of cortical plasticity in adult primates following brain injury.

PROJECT DESCRIPTION:

Previous work in our laboratory has shown that the amygdala and hippocampus are critical for both visual and tactual memory. The route by which visual information gains access to these structures has been well documented. In brief, a series of cortical fields beginning in striate cortex and progressing through prestriate cortex to inferior temporal cortex and from there to the amygdala and hippocampus, have been shown to be critical for the learning and memory of visual information. But while the cortical pathway for transmitting visual information to these limbic structures is well understood, the comparable somatosensory pathway, if one exists, is still uncertain. We are therefore trying to determine (a) the most direct anatomical route by which somatosensory information could reach the amygdala and hippocampus, (b) the physiological dependency of one cortical area on another along this route, (c) the processing of information along this pathway, and (d) its role in somatosensory learning and memory. If such a functional pathway could be identified, it would provide strong support for the view that there is a common plan of organization across all the modalities for the cortical processing, storage, and retrieval of sensory information.

Anatomical studies

Corticocortical connections. Anatomical studies have indicated that visual information is transmitted ventrally to the temporal lobe via a series of relays in prestriate areas. Specifically, V1 projects to V2 (area OB), V2 projects to areas V3 and V4 (both fields are part of area OA), and V4 projects to the inferior temporal areas TE and TEO. Area TE of inferior temporal cortex is the last cortical visual processing station in the sequence, and this area projects in turn to the amygdala (directly) and to the hippocampal formation (indirectly via entorhinal cortex). Also, area TE is totally dependent upon input from the more posterior areas in the chain for visual activation of its neurons. This pathway, which remains modality specific throughout its neocortical extent, has been shown to be important for the visual recognition and identification of objects.

By contrast, a second cortical visual pathway is thought to be important for visuospatial perception. This is a dorsally directed cortical pathway that begins in V1, passes through prestriate visual areas, then courses through posterior parietal cortex and then on to the cingulate cortex before finally reaching the amygdala and hippocampus. Much less detail is known, however, about the anatomical and physiological relationships between the areas comprising this pathway and the behavioral consequences of interrupting this pathway by lesions.

To determine whether the first type of pathway described above might be a common feature of organization for learning and memory in all of the sensory modalities, we turned to the somatosensory system since more is known about the cortical organization of this sensory modality than of any other except vision. Our experiments indicated that the fields comprising postcentral somatosensory cortex (areas 3a, 3b, 1, and 2) are richly interconnected with each other and that these connections link corresponding representations in the different fields. In addition these fields have further connections with

areas outside the postcentral cortex. On the basis of the laminar patterns of these various connections, each was designated as a forward projection (layer III to layer IV) or as a backward projection (layer V to layer I) by analogy to similar designations in the visual system. From this analysis, we were able to identify two major pathways for the flow of somatic information out of postcentral cortex.

The first cortical pathway is directed ventrally and begins with the primary cortical receiving area for tactual information, area 3b. Area 3b projects forward to area 1 and less densely to area 2. The densest cortical projection from each of areas 3b, 1 and 2, however, is to layer IV of SII cortex. SII then projects in a forward manner to the granular and dysgranular fields of insular cortex, and, finally, the pathway proceeds from the fields of the insula to the amygdala and indirectly to the hippocampus through perirhinal cortex. We have been slowly accumulating additional physiological and behavioral evidence that this pathway in the somatosensory system may be analogous to the ventrally directed pathway in the visual system.

The second pathway is a dorsally projecting one, again with area 3b projecting forward to area 1, and less densely to area 2. Area 1 in turn projects forward to area 2 and to a specialized cutaneous portion of area 5. Area 5 in turn projects to area 7b of posterior parietal cortex. Each of the areas receiving a forward connection projects with a backward connection upon the cortical area that gave rise to the forward one. It is not yet known whether this dorsally directed somatosensory pathway eventually has connections with the limbic system, as does the dorsal visual pathway.

Electrophysiological studies

Receptive fields of SII neurons following ablation of postcentral cortex. It had previously been assumed that the primary thalamic nucleus for tactual information (the ventroposterior nucleus, or VP) supplied the major activating input for both postcentral cortex and SII. However, our anatomical studies summarized above suggested instead that SII cortex may be receiving its somatosensory input from postcentral cortex, rather than directly from the ventroposterior nucleus of the thalamus. To examine this possibility, we recorded single- and multi-unit activity from the SII region in 10 hemispheres of 6 macaques (4 *Macaca mulatta* and 2 *Macaca fascicularis*) anesthetized with a mixture of halothane and nitrous oxide. The electrode penetrations were placed 0.5-1.0 mm apart in a rectangular grid across the entire extent of SII, and neuronal responses were sampled at 200 μ m intervals through the depth of this cortex. The receptive fields of the neurons at the recording sites were determined by applying tactile stimulation at different locations on the contralateral body surface. Of the 10 hemispheres studied, 5 were intact and 5 had received lesions 6-8 weeks earlier of selected portions of the body representations in the postcentral strip.

In the intact hemispheres, receptive fields of neurons in SII were readily found for tactile stimulation of all contralateral body parts, with the majority of the fields representing loci on the glabrous and hairy surfaces of the hand. By contrast, in recording sites through SII of hemispheres in which the postcentral hand representations had been removed, no receptive fields

could be found for tactile stimulation of the glabrous surface of the hand, and only a few were found that included the hand's hairy surface. Yet there was no difficulty in recording responses in the experimental hemispheres to stimulation of all other body parts, indicating that the near absence of a hand representation in SII was not due simply to a general depression of the SII cortex. The functional dependency on postcentral cortex that was demonstrated for the SII representation of the hand held also for the SII representations of other body parts. For example, in recording sites distributed through the SII region in a case with a postcentral removal that spared only the hand representation, all of the receptive fields found were confined to the hand. Similarly, in recording sites through SII in a case with a total removal of the postcentral strip, no neuronal activation was observed from tactile stimulation of any body part. In short, the elimination of any representation in the postcentral cortex eliminated it also in SII. The results thus support the proposal derived from our anatomical studies that SII depends on the postcentral strip for its somatic activation and thus could well occupy an intermediate position between the postcentral cortex and the insula in a sequential cortico-limbic pathway for touch.

Postoperative cortical plasticity. Another, unexpected result from the foregoing electrophysiological work was the finding that the SII region undergoes major functional reorganization following removal of portions of postcentral cortex. As indicated above, removing the representations of a body part in postcentral cortex results in the failure to record somatically driven responses in the representation of the corresponding body part in SII. Interestingly, the SII tissue in question does not remain silent; instead, representations of different body parts in the adjacent portions of SII expand to occupy the partially deafferented cortical zone. For example, following a lesion of the postcentral representation of the hand, there is a greater probability of recording responses in SII to stimulation of the foot. In other words, the areal extent of the foot representation increases to occupy part of the former hand region (indeed, the reorganizational changes occur over a distance of 4 or more millimeters of cortex), and, similarly, the rest of the body representation in SII also expands. These findings provide evidence for a previously unrecognized degree of cortical plasticity in adult primates. The results thus require major revisions of current theories, which tend to confer static properties on cortical maps.

Receptive fields of insular neurons. In another recording project, we are mapping the somatically responsive portions of the insular cortex, which our anatomy shows receives a dense input from SII, in an attempt to determine the somatotopic organization and response properties of cells in this region. Because anesthetics are known to depress higher order sensory cortical areas such as the insula, this study must be performed in awake responding monkeys trained to sit in a primate chair and to allow gentle tactile stimulation of their bodies. The preliminary results indicate that receptive fields for insular neurons are typically very large, usually bilateral, and are modality specific. Our recordings have also revealed that there is at most only a rough somatotopic organization within the granular insular cortex, with the face and intraoral regions being represented rostrally, and the rest of the body being represented more caudally. These receptive-field properties are clearly analogous to those of neurons in visual area TE, in that they too are

modality-specific, are large and bilateral, and have poor topographic organization. Our data are thus consistent with the notion that insular cortex serves as a final link in a somatosensory-limbic pathway just as area TE does in the visual-limbic pathway.

Neurobehavioral studies

We have been using behavioral techniques to examine the effects of removing the insula on tactile object recognition and have postoperative results from four animals indicating that bilateral insula lesions cause a tactile recognition deficit. This preliminary finding is consistent with the suggestion that insular cortex acts as a final link in a parieto-insulo-limbic pathway for somesthesia, analogous to the occipito-temporo-limbic pathway previously described for the visual system. This preliminary result is particularly exciting, for, if it is upheld, the project has the potential of revealing a mode of sensory-limbic interaction that is common to memory formation in all sensory modalities.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

The data from these projects are providing us with the first comprehensive view of the entire somatosensory system as well as of its connections with the limbic memory system. Furthermore, the studies have suggested remarkable parallels between the organization of the somatosensory and visual systems, and imply that similar mechanisms of perception and memory may operate within both. These projects are yielding fundamental insights into how the cerebral cortex processes and stores sensory information by uncovering mechanisms that may well be common to all sensory modalities. Finally, we have demonstrated a previously unrecognized degree of post-injury cortical plasticity in the adult macaque with the exciting discovery that SII cortex undergoes extensive functional reorganization following damage to primary sensory cortex.

PROPOSED COURSE OF RESEARCH:

More research is required to gather further evidence for, or against, the possibility that the ventrally directed pathway in the somatosensory system is analogous to the ventrally directed one in the visual system. In addition, we plan to begin research projects designed to determine if the dorsally directed somatic pathway might have a role in tactual spatial perception, by analogy to the role of the dorsal visual pathway in visual spatial perception.

PUBLICATIONS:

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Garraghty, P.E., Pons, T.P., Huerta, M.F., and Kaas, J.H. Somatotopic organization of the third somatosensory area (SIII) in cats. Somatosens. Res. 4: 333-357, 1987.

Kaas, J.H. and Pons, T.P. The somatosensory system of primates. In H.P. Steklis (Ed.): Comparative Primate Biology, Vol. 4, Alan Liss, New York, (in press)

Pons, T.P., Garraghty, P.E., Friedman, D.P., and Mishkin, M. Physiological evidence for serial processing in somatosensory cortex. Science 237: 417-419, 1987.

Pons, T.P., Wall, J.T., Garraghty, P.E., Cusick, C.G., and Kaas, J.H. Consistent features of the representation of the hand in area 3b of macaque monkeys. Somatosens. Res. 4: 309-331, 1987.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02038-05 LN

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Ontogenetic development of cognitive memory and habit formation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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COOPERATING UNITS (if any)

National Institute on Drug Abuse

LAB/BRANCH

Laboratory of Neuropsychology

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland

TOTAL MAN-YEARS:

2.25

PROFESSIONAL:

1.0

OTHER:

1.25

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Cognitive memory and habit formation are two qualitatively different learning processes based on separate neural systems, a cortico-limbic and a cortico-nonlimbic system, respectively. To see how emotional and social behavior develop in animals whose infantile global amnesia might persist from infancy through adulthood, we have prepared monkeys with neonatal limbic lesions and followed their behavioral development. Animals with neonatal removal of cortical area TE, a higher-order visual station linked to both learning systems, serve as controls. The results indicate that neonatal TE lesions leads to a transient impairment of habit formation three months later (compared to permanent impairment seen with the same lesion in adults), whereas both neonatal and adult limbic lesions leave habit formation intact. Interestingly, data on both normal and operated infants suggest that development of the nonlimbic habit system is sexually dimorphic, and that this is due to the high testosterone levels present in male infants before and shortly after birth. At ten months of age, the infants with limbic lesions show impairment in memory formation, whereas the operated controls show significant functional sparing (compared to those that received the same lesions as adults). These findings point to greater compensatory potential after neonatal cortical than after neonatal limbic removals, indicating that association areas of the cortex are immature at birth, and may thus possess greater plasticity than limbic structures. Direct evidence of neocortical immaturity in the macaque has been provided by our neurobiological studies on opiatergic and cholinergic receptor distribution and on metabolic activity. Finally, early damage to the limbic memory system produces later socio-emotional abnormalities that are similar in many respects to the behavioral syndrome seen in autistic children.

PROJECT DESCRIPTION:

Findings from studies of the effects of lesions in adult monkeys suggest that cognitive memory and habit formation are qualitatively different retention processes based on separate neural mechanisms. The cognitive memory system, which serves both recognition and associative memory, utilizes a cortico-limbo-diencephalic circuit. By contrast, the habit system, which mediates retention of stimulus-response connections, probably depends in large part on a cortico-striatal system. Our recent studies of behavioral development in infant monkeys have suggested that these two systems are developmentally dissociable, in that the nonlimbic habit system appears to mature considerably earlier than the limbic memory system. On the evidence that the limbic memory system is essentially nonfunctional in infants, we have prepared monkeys with neonatal removal of this system in an attempt to see how emotional and social behavior develop in animals whose amnesia might persist from infancy through adulthood. In addition, since the ontogenetic development of habit formation appears to be sexually dimorphic, we have initiated a study of the neuroendocrinological substrate of that dimorphism. In tandem with these developmental studies of behavior, we continue to map the distribution of opiate and muscarinic cholinergic receptors in the brain of the developing rhesus monkey.

Experiment 1

To date, eight infant rhesus monkeys received damage to the limbic system (i.e. amygdalo-hippocampal complex) and eight others, which served as control animals, received damage to the anterior part of inferior temporal cortex (i.e. cytoarchitectonic area TE, a higher-order station of the visual system). These monkeys were age-matched with thirteen normal animals and have already undergone some testing for social behavior and learning abilities. We are currently following the behavior of these animals from birth through early adulthood in order to assess the effects of neonatally induced amnesia on (1) the maturation of cognitive functions and skill learning, as measured by a variety of visual memory, problem solving, and habit formation tasks, and (2) the development of emotional and social behaviors, as measured by interactions with familiar vs. unfamiliar and normal vs. operated monkeys of both sexes and various ages, and by reactions toward familiar vs. unfamiliar and emotionally neutral vs. emotionally challenging environments and stimuli.

Maturation of skill learning and cognitive functions in early infancy. The results so far indicate that, at three months of age, neonatal ablation of area TE leads to a transient impairment in habit formation (compared to the permanent impairment seen with the same lesion in adults), whereas limbic lesions in both infants and adults leave habit formation intact. Interestingly, data from both the normal and the operated infants suggest that ontogenetic development of the habit system is sexually dimorphic, this system maturing earlier in females than in males (see Experiment 2). At ten months of age, the infants with limbic lesions are severely impaired in cognitive memory, whereas the operated controls show significant functional sparing of this ability (compared to the animals given TE lesions as adults). Thus, the mild impairment seen in infants with area TE lesions in recognition memory points to greater compensatory potential after neonatal cortical than after

neonatal limbic removals. The results are consistent with the notion that association areas of the cortex are less mature at birth, and may thus possess greater plasticity, than limbic structures. Direct evidence of neocortical immaturity in the macaque has been provided by our neurobiological studies showing that (a) adult levels of metabolic activity in visual association cortex are not reached until about 4 months of age (see Experiment 3), and (b) the distribution of both opiate and muscarinic cholinergic receptors is adult-like at birth in subcortical structures and allocortical areas but is not yet fully developed in neocortical areas, particularly the association cortex (see Experiment 4). These behavioral and neurobiological findings suggest that the reduced recognition ability in infant monkeys and perhaps, by extension, infantile global amnesia in this species are due more to slow maturation of the cortical association areas than to neonatal immaturity of the limbic system.

To investigate whether the severe impairment in cognitive memory in infants with the limbic lesions is due, as it is in adults, to the combined damage of the amygdala and the hippocampus and not to the damage of either limbic structure alone, we have prepared new groups of infant monkeys who received damage to either the amygdala or the hippocampus. These monkeys were age-matched with normal infants and were tested in exactly the same way as the infants with combined limbic lesions. The findings indicate that, as in adults, damage to the hippocampus or the amygdala alone do not yield marked impairment in the visual recognition task. In fact, whereas both amygdalar and hippocampal lesions in adulthood yielded a mild memory loss, only the neonatal amygdalar lesions produced a deficit equivalent in magnitude. The neonatal hippocampal lesions by contrast left this ability intact.

Some of the first infants to receive neonatal lesions are now five years old and are being retested on the behavioral tasks. This will allow us to determine what changes have taken place in their cognitive and noncognitive learning abilities since they were last tested, which was when they were about a year of age.

Development of emotional and social behaviors in early infancy. The slowly accumulating data of this long-term neurobehavioral study suggest that, compared to intact infant monkeys and others with neonatal cortical lesions, monkeys with neonatal limbic lesions show numerous socio-emotional abnormalities such as increased locomotion, decreased manipulation of toys, towels, and cage surfaces and attachments, increased withdrawal from social contacts, and increased finger-sucking. All of these abnormalities are reminiscent of those seen in young autistic children. And, indeed, our results, combined with Kempers's recent report of neuropathology in the amygdala, hippocampus, and cerebellum of an autistic man, support the view that early dysfunction of the limbic system is one cause of infantile autism. Although amygdalar damage by itself is associated with changes in emotional and social behavior in adult monkeys, the socio-emotional abnormalities we observed in the developing infants with combined amygdalo-hippocampal lesions was not attributable to amygdalar damage alone. Rather, the full-fledged syndrome described above was fractionated by partial limbic lesions. Specifically, the findings indicate that whereas amygdalar lesions in infants produce the same behavioral abnormalities that they do in adults, neonatal

hippocampal removals also induce socio-emotional abnormalities, a finding that has not been reported before at any age. Together these experiments are providing evidence that the amygdala and hippocampus are each components not only of a limbic-thalamic system serving cognitive functions but also of a limbic-hypothalamic system serving emotional functions. Though much more testing over a much longer time course is necessary, it is becoming clear that the same neonatal damage that leads to a severe cognitive memory disorder can have extremely serious consequences for personality and social development, in part perhaps because the cognitive memory disorder is present from infancy onward, but also because of the direct effect of the limbic lesions on mechanisms of emotionality.

To compare the socio-emotional abnormalities in the neonatally operated animals with those observed in autistic patients, we will retest the animals as they reach adulthood and analyze in detail their individual and social behavior for characteristic signs of autism such as disinterest in initiating play when placed in a group, lack of separation anxiety, fearful responses to novelty and environmental complexity, and unusual food preferences.

One way to measure separation anxiety in monkeys is to record "isolation" calls. These calls serve to reestablish contact between a mother and her offspring, as well as between peers raised together. Cerebral ablation studies in squirrel monkeys have demonstrated that these calls can be eliminated by lesions of the cingulo-thalamo-limbic system. In collaboration with Dr. John D. Newman from the Laboratory of Comparative Ethology (NICHD), we have begun to record "isolation" calls in normal infants and infants who received neonatal amygdectomy. Our preliminary findings indicate several alterations in the production of isolation calls in infants with amygdectomy, possibly reflecting a lack of separation anxiety in these animals.

Experiment 2

The cortico-nonlimbic habit system becomes functional in female infants earlier than in males. The evidence for this sexual dimorphism comes from the findings that female infants learn visual discrimination habits faster than males, and neonatal ablation of area TE impairs learning of female but not of male infants. These two functional differences, which are apparent only when the monkeys are less than about six-months-old, indicate that area TE or its striatal targets matures faster in females than in males. In a recent neuroendocrinological study, we have further demonstrated that the high levels of testosterone present in male infants before and shortly after birth are probably responsible for this sexual dimorphism, since a significant correlation appeared in male infants between their testosterone levels and learning scores (the higher the level the poorer the scores), and also because orchietomy in male infants actually speeded their rate of habit formation to equal that of females. We extended this experiment by adding a group of infant females that were ovariectomized at birth and then received either testosterone propionate (TP) or dihydrotestosterone (DHT), the two active forms of testosterone. The treatments proved to be ineffective, but perhaps only because they were given after the period in which testosterone exerts its organizational effect on the brain. To test this proposal, we are currently

adding to the experiment a group of infant females that are receiving testosterone treatments prenatally. If the learning ability of this new group of females were to be developmentally delayed as it is in normal male infants, it would provide the first direct experimental evidence that gonadal hormones in the primate fetus influence the maturation of a telencephalic system important for learning.

Experiment 3

The 2-deoxyglucose method was applied postoperatively to a series of infant monkeys that had received optic tract section combined with forebrain commissurotomy at 1 day, 1 week, and 1, 2, 4, and 6 months of age. In all visual cortical areas of the intact hemisphere, LCGU was lowest in the youngest subjects, peaked at 4 months, and then declined in the 6-month-old subject to levels found in adults. This finding is consistent with behavioral data indicating that adult levels of visual object recognition probably do not develop until about this time and that neonatal removal of inferior temporal cortical area TE produces a significant sparing of this function. But a sex-related factor could also have affected the results, inasmuch as the peak metabolic activity seen at 4 months of age was found in a female infant, whereas the lower levels of metabolic activity seen both at two months and at six months of age were found in males. It is therefore possible that the results reflect the sexually dimorphic maturation of the cortico-nonlimbic visual pathway described in Experiment 2. To test this possibility, and also to complete the developmental sequence, three-month-old male and female infants have been added to the study.

Experiment 4

Direct evidence that limbic and cortical systems differ in rate of development has been provided by our developmental neurobehavioral studies. In previous experiments, we found that the distribution of opiate and muscarinic receptors in the macaque brain is adult-like at birth in limbic and striatal structures but is not yet fully developed in neocortical areas. Thus, like many other aspects of neocortical maturation, opiate and cholinergic mechanisms continues to develop postnatally. We are continuing to follow the developmental course of these mechanisms by examining them in infants of different ages.

Experiment 5

Visual recognition in adult monkeys is critically dependent on a neural system that includes both the inferior temporal cortical area TE and limbic structures. As described in Experiment 1, however, infant monkeys given lesions of area TE show significant sparing of visual recognition ability. Through the use of behavioral, anatomical, and metabolic mapping techniques, we will test two different hypotheses that could account for this sparing of function, namely, (1) that afferents from other visual cortical areas innervate the limbic tissue that was deafferented by the area TE lesions, and (2) subcortico-limbic interaction substitutes for the absent cortico-limbic interaction.

Experiment 6

In collaboration with investigators from The Johns Hopkins School of Medicine, we have begun an assessment of the decline of learning and memory in aged monkeys. We found impairments in a wide variety of learning and memory tasks, suggesting that there is widespread cerebral dysfunction in aged rhesus monkeys, probably due to the vulnerability of multiple neural systems to increasing age. At the same time, these learning and memory impairments vary widely from one aged animal to another within a given task, and there is no correlation in degree of impairment for a given aged animal across tasks, suggesting that the distribution and density of age-related neuropathological changes is likely to vary considerably from animal to animal. This possibility will be investigated directly through postmortem localization of neuritic plaques and depletion of cholinergic and other neurotransmitters. Thus, the use of the aged nonhuman primate as a model for human aging will provide important information regarding the relationship between age-related cognitive changes and pathological alterations in the brain.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Developmental studies of the effects of early brain damage are of great importance for the assessment and understanding of those errors of central nervous system maturation that cause children to become autistic, dyslexic, learning disabled, or mentally retarded. This project will provide the first comprehensive evaluation of the social and cognitive development of monkeys suffering from an amnesia induced by limbic lesions early in infancy as compared to those rendered amnesic in adulthood, i.e. after memories have been formed and consolidated in cerebral tissue outside the limbic system. In addition, comparison of the effects of early and late cortical and subcortical lesions will help answer whether or not compensatory mechanisms always operate to promote recovery from early brain injury. Our preliminary results suggest otherwise. In assessing the effects of early and selective temporal-lobe damage on infant, juvenile, and adult behavioral patterns, this project will help to evaluate two provocative proposals from the clinical literature: (a) that early dysfunction of the limbo-thalamic memory system is one cause of childhood autism, a syndrome characterized by dramatic social and emotional disturbances not seen in adults with the same neuropathology; and (b) that the reason a pure case of global anterograde amnesia like the one seen in adults has never been reported in a child is that the clinical picture of an amnesic child, being overlaid with autism, is entirely different from the clinical picture of an amnesic adult. In addition, whereas our neurobiological studies indicate how brain maturation normally progresses postnatally, our neuroendocrinological studies demonstrate how the perinatal hormonal environment may influence brain maturation and, consequently, the development of cognitive functions. Finally, the studies in young adult monkeys together with those in normal aged animals are providing the anatomical and chemical basis for understanding the memory disorders in humans that accompany cerebrovascular and other cerebral accidents and diseases as well as normal aging.

PROPOSED COURSE OF RESEARCH:

Our goal is to continue examination of the effects of neonatal limbic lesions on social and emotional behavior as well as on cognitive memory and habit formation at several periods throughout development from infancy to adulthood in order to test whether such a preparation does indeed provide an animal model of childhood autism. With the discovery of an important sexual dimorphism in the development of the habit system, we plan to pursue studies aimed at determining the neuroendocrinological substrate of that dimorphism. We shall also pursue studies to determine how recognition memory measured by preferential viewing differ from recognition memory measured by problem solving. This will help determine which capacities of the memory system appear late in ontogenetic development and, by implication, whether the phenomenon of infantile amnesia might be due to the absence of a fully functional cognitive memory system in early childhood. We shall continue our attempts to follow the development of neurochemical receptors in infant monkeys. In addition, new experiments have been initiated to study the neural mechanisms by which visual object recognition can develop in the absence of higher-order areas of the visual pathway. Finally, we will pursue our longitudinal study on learning and memory decline in normal aging.

PUBLICATIONS:

Presty, S.K., Bachevalier, J., Walker, L.C., Struble, R.C., Price, D.L., Mishkin, M., and Cork, L.C. Age differences in recognition memory of the rhesus monkey (Macaca mulatta). Neurobiol. Aging (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02039-05 LN

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology of cognitive memory and habit formation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Evidence from patients with Alzheimer's disease suggest that the basal forebrain cholinergic system plays an important role in memory. In support of this proposal, we have found impaired visual recognition memory in monkeys with lesions to the major nuclei of this system. We have found further that recognition memory in normal monkeys can be improved by the cholinesterase inhibitor physostigmine and impaired by the cholinergic muscarinic-receptor blocker scopolamine. In addition, our results indicate that scopolamine acts at a very early stage of memory, preventing information from entering even into an immediate store.

Based on previous results indicating that THC may be exerting its effects through an action on the limbic system, we administered this drug to monkeys performing spatial reversal, a task known to be sensitive to hippocampal damage. Doses equal to or even greater than those that impaired recognition memory did not affect performance on this task, though performance did appear to be affected several days after the last dose of THC, raising the possibility of a delayed abstinence effect.

In a series of experiments on habit formation, we administered the dopaminergic-neurotoxin MPTP to monkeys, but failed to show learning impairments at doses that did not also impair motor function. Administration of MPTP did make the animals more sensitive to the disruptive effects of scopolamine, however, suggesting that the cholinergic-dopaminergic balance in the neostriatum had been compromised.

PROJECT DESCRIPTION:

The cholinergic system is now suspected to play a critical role in mnemonic processes both in humans and in primates. Our work during the past year has continued to concentrate on identifying the processes and functions in which acetylcholine influences memory in primates. We previously showed that scopolamine, a muscarinic-cholinergic receptor blocker, impairs recognition memory in monkeys performing a delayed nonmatching-to-sample task with trial-unique objects. In addition, we showed that scopolamine produced greater impairments when administered before, rather than after, the acquisition trials, suggesting that this drug has mainly an anterograde effect, influencing storage more than retrieval.

Experiment 1

We previously showed that recognition memory impairments could be produced in monkeys by combined but not by separate neurotoxin-induced lesions of the three major nuclei of the basal forebrain cholinergic system. In addition, these animals showed an altered sensitivity to cholinergic drugs compared to normal animals. The cells of the basal forebrain cholinergic nuclei, particularly those constituting the nucleus basalis of Meynert, the major source of cholinergic innervation of the cortex, form an extremely complex shape that renders them very difficult to locate and damage by standard stereotaxic methods. In our original studies, we used electrophysiological recording to identify the boundaries of the anterior commissure (AC), which then served as a reference for locating the basal forebrain nuclei. Although we obtained significant functional impairments with this method, the lesions were not complete and were highly variable. We are therefore planning a repeat experiment in which we will try two new methods for producing lesions of this system. One method will involve direct visualization of the dorsal surface of the AC which we will then use to calculate the areas into which the neurotoxin will be injected. The second method involves the use of magnetic resonance imaging (MRI) to locate the AC and surrounding structures. We have designed and built a specially modified stereotaxic frame that will permit use with the MRI scan. The initial scans have shown even more detail than anticipated, so that this method promises to be useful not only for this study, but for others as well.

Experiment 2

During the past year we have continued to develop an automated testing apparatus that is allowing us to study drug effects on memory in monkeys more rigorously than previously possible. The apparatus consists of a color monitor that is outfitted with a touch-sensitive glass screen, both of which are connected to a personal computer. We have designed and written programs that utilize the capacity of the computer to generate and to present to the monkeys a wide variety of graphic symbols in a series of experimental paradigms.

In our first use of the apparatus last year, we examined the effects of scopolamine on delayed nonmatching-to-sample (DNMS) with a small set of symbols, a test of recency memory. In this task, a single symbol (a 25 mm

colored square) was presented first (acquisition) and the monkey was required to touch the screen within the boundaries of the symbol. Then, after a delay of 0, 1, 3, 10, 30, or 60 sec, the original symbol was presented with another symbol of a different color in a choice trial (test), and the monkey was rewarded for touching the novel symbol. The colors of the symbols and the delay interval were randomly selected by the computer, which recorded the position of the touch, the symbol selected, and the reaction time to touch the screen during correct and incorrect choices for every trial. In this initial study, we obtained forgetting curves, that is, a decrease in the number of correct choices as the delay interval increased, during control sessions. In addition, we found that scopolamine, in doses known to impair performance in the Wisconsin General Testing Apparatus (WGTA), also impaired performance in the automated apparatus. More importantly, we found that scopolamine produced its greatest effect in the interval between 0 and 1 sec. That is to say, there was no effect of scopolamine at the 0 sec delay, but by 1 sec, the number of correct choices was significantly decreased. The forgetting curve from 1 to 60 sec paralleled that of the control sessions, suggesting no further decrement in performance with increasing delay. These results, when combined with our previous findings that the effects of scopolamine are mainly anterograde, suggested that scopolamine exerts its effects at a very early stage of memory, preventing information from entering even into an immediate store.

Experiment 3

In our next series of studies on the effects of cholinergic drugs on memory, we modified the DNMS procedure in the automated apparatus to more closely resemble that used in the WGTA, where trial-unique objects are used. In this new test, the computer randomly generated a new series of symbols in a variety of different shapes and colors, such that the animal never saw the same symbol more than once each session. The monkeys were able to perform this task more accurately than they were the recency task. Although the animals did more poorly with long delays than with short ones, they were nonetheless able to perform at a level of nearly 90% correct choices at the 60-sec delay, a value approximately 15% greater than at the comparable delay on the recency test. Although scopolamine administration (10.0, 17.8, and 32.0 ug/kg) produced dose-related impairments in DNMS with a large sample set, the impairment was less than that observed with the small sample set, suggesting that recognition memory is less susceptible to disruption than recency memory.

In addition to the DNMS task, each session also included a new task, called recognition scan. In this test, the screen is divided into nine sectors arranged in a 3 X 3 matrix. On the first trial, a symbol is randomly located in one of the nine sectors, which the animal must then touch for reward and for the task to continue. After a 5-sec interval in which the screen is dark, the original symbol, now in a randomly selected new location, and a novel symbol are projected on the screen. In this and all subsequent trials, the animal must touch the new symbol to obtain a reward. If the animal correctly touches the novel object on the screen, the procedure is repeated after a time-out, this time with three symbols, the two previously seen, plus a new, previously unseen, symbol. With each new symbol addition, the position of all of the symbols are randomly relocated to one of the nine positions on the

screen so that the animal cannot rely on spatial cues to determine which symbol has been newly added. A trial is continued until the animal has either correctly touched all nine symbols or has incorrectly touched a previously seen symbol. Thus, we can determine the average number of correct responses, or list length, that the animal can recognize. Three different versions of this task are used in a session. In the first, the symbols differ in shape, but are the same color on each trial. In the second, the symbols are the same shape, but are different colors. In the third task, the symbols change in both dimensions. Under control conditions, the animals correctly recognized an average of 7 out of 9 symbols correctly when both shape and color were varied, 5.7 when shape was varied, and 5.1 when color was varied. On the recognition-scan test, scopolamine produced dose-related impairment on all three measures, although the degree of impairment again was related to set size. That is, 10 ug/kg of scopolamine significantly reduced the mean list length when the symbol varied only in color, a condition in which the set size was limited to 9, but had no effect when both symbol and color were varied, a condition in which set size was nearly unlimited. Administration of the cholinesterase inhibitor physostigmine produced the opposite effect, i.e. it improved performance, and, in this case, the smaller the set size, the greater the improvement. Taken together, these results replicate our original findings, obtained in the WGTA, that cholinergic agonists and antagonists improve and impair recognition memory, respectively, and do so in the same dose range in both procedures.

Experiment 4

We have continued our collaboration with the National Institute of Drug Abuse on the cognitive effects of delta-9-tetrahydrocannabinol (THC), the active ingredient of marijuana. Previously, we reported that THC impaired recognition memory, but not habit formation, suggesting a possible selective action of the drug on limbic structures. Indeed, a number of reports by others suggested that THC may have a deleterious effect on the hippocampus specifically. To examine this possibility further, we next studied the effects of orally administered THC on spatial reversal, a task known to be especially sensitive to hippocampal lesions in monkeys. Daily oral doses of 4, 8, or 16 mg/kg failed to affect task performance, although all animals showed overt behavioral signs of marijuana intoxication. Of interest, however, was an impairment in control performance that occurred approximately 7 days after the last dose of either 8 or 16 mg/kg. We had previously reported a delayed abstinence syndrome in DNMS performance following 21 days of 4 mg/kg of THC that was similar to that observed here. Because recognition memory is known to depend equally on the hippocampus and the amygdala, we next examined the effects of THC on object reversal, a task in which impairments are observed following lesions of the amygdala in monkeys. The same four monkeys used in the spatial reversal task were trained to displace one of two visually different objects to a high criterion of 90% correct choices in one session. In the task, the left-right position of the positive object on the lateral food wells of the test tray is varied in a pseudorandom sequence. After reaching criterion, the animal is rewarded only for displacing the originally negative object, and so on for several reversals. Again, however, doses of 4, 8, or 16 mg/kg of THC failed to impair performance, and signs of an abstinence syndrome were noted, although these were not as severe as those

during place reversal. Apparently, either dysfunction in limbic structures is not responsible for the recognition impairment produced by THC or the reversal tasks were not sensitive enough to detect the dysfunction. This issue will be examined further with newly designed tests.

In a separate study, we examined the effects of orally administered THC (2, 4, or 8 mg/kg) on performance of the color memory task in the automated testing apparatus. THC, like scopolamine, impaired performance of this task in a dose-related manner. In contrast to scopolamine, however, which produced its greatest effect between 0 and 1 seconds, THC produced its greatest impairment at the longer delays (30 and 60 sec). Thus, although THC and scopolamine produce equivalent impairments in short-term recognition, they do so starting at different delays, suggesting that these two drugs have different mechanisms of action.

Experiment 5

In the past year we have continued our study of the contributions of the neostriatum to habit formation. We had previously administered 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that selectively destroys the nigrostriatal dopamine system when given in high doses, to four monkeys. This drug has been shown to impair motor function permanently in much the same way as that observed in Parkinson's disease. The monkeys were tested both for motor function as well as concurrent discrimination learning with 24-hour intertrial intervals, a measure of their ability to acquire habits. One pair of monkeys was given MPTP in a series of small doses at approximately one-month intervals, such that any effects on learning unaccompanied by motor deficits could be identified. Only when the cumulative dose of MPTP exceeded 4.0 mg/kg (approximately twice that required to produce motor impairment if given in higher doses or at shorter intervals), did we observe learning impairment. At these dose levels, however, motor impairment was also observed. When testing was continued following termination of drug administration, both learning and motor impairments dissipated. In the other pair of monkeys, MPTP was administered in higher doses and at shorter intervals (0.5 mg/kg, once per week for three weeks). Both of these monkeys developed severe movement disorders that persisted for two to three weeks before gradually resolving. Only one of these monkeys showed evidence of a learning impairment after this dosing regimen, although when a fourth dose of MPTP was administered one month later, neither animal showed a learning deficit. Indeed, both monkeys appeared to be less sensitive also to the drug's effects on motor function.

Because of the known interaction of the cholinergic and dopaminergic systems in the striatum, we tested the effects of scopolamine in these animals to determine if they might be hypersensitive to blockade of the cholinergic system after the dopaminergic system was compromised. We had previously shown in normal monkeys that a high dose of scopolamine (32 ug/kg) slowed learning rates only slightly. In the two monkeys given the protracted MPTP regimen, this same dose of scopolamine was administered prior to each daily session. During the first scopolamine series, one monkey, which normally attained criterion performance in six days, failed to learn within the 40 day period allowed. When drug administration was terminated, this animal was again able

to reach criterion within six days. The second animal, which like the first averaged approximately six days to learn under control conditions, was able to learn the discriminations during scopolamine testing, but required 25 days to do so. Motor function tests in these two animals showed an initial disruption, which recovered at a faster rate than did the learning impairment. This procedure was repeated on two subsequent series and both monkeys appeared to be less sensitive to disruption by scopolamine than they were initially, although both still required more than 20 days to learn the discriminations. The two monkeys given the accelerated MPTP dosing regimen were even more sensitive to the scopolamine-induced learning impairments. One animal failed to reach criterion within 40 days during the three scopolamine test series; the second animal failed on the first series, but then learned in 22 and 35 days on the next two series. Both monkeys also showed severe impairments in motor function during scopolamine testing. Further, their motor performance did not improve as it did in the first two animals. These results suggest that MPTP administration in all four animals increased their sensitivity to the disruptive effects of scopolamine, and that the combination of treatments affected motor performance and habit formation simultaneously.

During the next few weeks, we plan to make further examination of the dopaminergic function of these four monkeys. Although they presently show no explicit effects of MPTP administration and, indeed, perform normally on all tests of motor function, the results just described for scopolamine challenge several months after the last dose of MPTP suggest that the dopaminergic system may have been damaged, but perhaps not sufficiently to either prevent recovery or compensation by remaining neurons. Since the dopaminergic system is known to compensate for neuronal loss up to a point, these animals may be more sensitive to challenge by other drugs or may even start to show deficits as they age, since dopaminergic activity is known to decrease with advancing age. By means of positron emission tomography (PET) and the use of radiolabeled compounds that specifically bind to the central D1 and D2 dopamine receptor subtypes, we may be able to identify and quantify the extent of damage to this system in our monkeys. In addition to the PET studies, we also plan to remove samples of cerebrospinal fluid from these animals to determine dopamine metabolite levels. These studies may suggest areas for further analysis by drug challenge.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Our results continue to provide convincing evidence that cholinergic mechanisms are critical for cognitive memory in monkeys. These mechanisms appear to play a more important role in storage than in retrieval and, further, the effect on storage occurs at a very early stage of processing. Our studies on the effects of MPTP on habit formation are inconclusive concerning the role of the dopaminergic system in this function, although our finding that motor functioning recovers even while the animals remain hypersensitive to disruption by scopolamine has important implications for understanding the neurochemistry and plasticity of this system. Finally, although our findings indicate that administration of high doses of THC does not directly impair performance on certain tests of limbic function, there is evidence that performance may be affected several days after the last administered dose, suggesting a delayed abstinence syndrome to the drug.

PROPOSED COURSE OF RESEARCH:

We will continue to evaluate the effects of both cholinergic and noncholinergic compounds on cognitive memory and habit formation in monkeys. In addition, we will continue to examine the role of the basal forebrain cholinergic system in memory following direct damage to this system and to examine the actions of drugs in monkeys with lesions of this system. We will expand our research into the role of other neurotransmitters and neuropeptides in mnemonic processes by examining the direct effects of these agents on memory, as well as their ability to block the now well-documented impairments produced by scopolamine. Finally, we will continue to study the effects of high doses of THC during and after periods of chronic drug administration.

PUBLICATIONS:

Aigner, T.G. and Mishkin, M. Naloxone improves recognition memory in monkeys. Psychopharmacol. (in press)

Aigner, T.G., Mitchell, S.J., Aggleton, J.P., DeLong, M.R., Struble, R.G., Price, D.L., Wenk, G.L., and Mishkin, M. Effects of scopolamine and physostigmine on recognition memory in monkeys with ibotenic-acid lesions of the nucleus basalis of Meynert. Psychopharmacol. 92: 292-300, 1987.

Wenk, G., Engisch, K., McCall, L., Mitchell, S., Aigner, T., Struble, R., Price, D., and Olton, D. [³H]Ketanserin binding increases in monkey cortex following basal forebrain lesions with ibotenic acid. Neurochem. Int. 9: 557-562, 1986.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02040-04 LN

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functional analysis of neurotransmitter systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	T.P. Pons	Guest Researcher	LN NIMH
Others:	M. Mishkin	Chief	LN NIMH
	D.P. Friedman	Project Officer	NRB NIDA
	J. Bachevalier	Visiting Scientist	LN NIMH
	L.G. Ungerleider	Research Psychologist	LN NIMH
	C.B. Pert	Chief, Sec. Brain Chemistry	NSB NIMH
	A. Routtenberg	Professor	Northwestern Univ.

COOPERATING UNITS (if any)

National Institute on Drug Abuse
Section on Brain Chemistry, NIMH
Northwestern University

LAB/BRANCH

Laboratory of Neuropsychology

SECTION

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00471-32 LPP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Heredity and Environment in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Allan F. Mirsky, Ph.D. Chief LPP, NIMH

COOPERATING UNITS (if any)

Institute for Research on Kibbutz Education, Haifa University, Israel; Hebrew University, Israel; Oranim Teacher's College, Israel; Bar Ilan University, Israel; University of Chicago, Illinois

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been composed of the following studies: (1) An intensive multi-disciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome; (2) Studies of Danish adoptees and their biological and adoptive families; (3) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel. We maintain contact with the quadruplets but have not pursued active studies with them during the past two years. The Danish adoptees are of continuing interest to us and we are preparing additional reports on factors involved in their psychiatric outcome. The Israeli children are the subject of intensive research efforts and we are currently conducting further behavioral and biological studies with them.

DescriptionA. Other Personnel

Loring Ingraham, Ph.D.	Staff Fellow	LPP, NIMH
Shaul C. Schlberg, Ph.D.	Clinical Psychologist Bar Ilan University	Israel
Sol Kugelmass, Ph.D.	Professor of Psychology Hebrew University	Israel
Joseph Marcus, M.D.	Professor of Child Psychiatry University of Chicago	Chicago, Illinois
Judith Shotten, M.A.	Psychiatric Social Worker	Israel
Eugene P. Tassone	Psychologist	LPP/NIMH
Olive W. Quinn, Ph.D.	Guest Researcher	LPP, NIMH
Patricia Lowing, Ph.D.	Private practice	Michigan
Deborah Levy, Ph.D.	Director Psychophysiology Lab, SUNY Stonybrook	New York

B. Objectives

The project is composed of the following studies: (1) An intensive multi-disciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. We are continuing our contacts with this family to see what happens in the clinical course of these women and to see how the course is related to earlier and to current life experiences; (2) Studies of adoptees and their biological and adoptive families in Denmark; (3) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

C. Major Findings

The objectives of this project are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

1. The Genain Quadruplets

Our recent studies of the Genain quadruplets are summarized in the annual reports of the previous four years. This year, we expect to see publication of two reviews of prior studies of these women, one in a monograph on aging and schizophrenia and the other to be included in a special issue of the Schizophrenia Bulletin dedicated to long-term follow-up studies in schizophrenia. This edition of the bulletin will be edited by Drs. William T. Carpenter and Thomas H. McGlashan. Dr. Olive Quinn, who is a Guest Researcher in the LPP, maintains contact with the Genains and will be a coauthor of the two reviews referred to above. Interest in the quadruplets continues to be high, as judged by the steady demand for photographs of them for psychology text books.

Some time ago, Dr. Deborah Levy visited the Genains and made smooth pursuit eye movement recordings of all four women in their home. The results

of these measurements have been incorporated in the Schizophrenia Bulletin chapter referred to above.

2. The Danish Adoptee Study--Reanalysis of the Data

Using data from Danish health records, in a now-classic study, Rosenthal, Kety and Wender compared the frequency of schizophrenia spectrum disorders in two groups of persons adopted in infancy or early childhood: those with a psychotic parent (index group) and those whose biological parents had never had psychiatric treatment (control group). Significantly more disorder was found in the index than the control group. We have recently completed a reanalysis of the extensive interview data collected on this group of subjects, focusing on stressful events during childhood and adult outcome. In a report we have prepared for publication, we present evidence that more stressful events were found in the childhood histories of index subjects who developed schizophrenia or schizotypal personality disorder (SPD) than in the histories of index subjects free from these disorders, and that greater number of stressors are associated with more severe outcomes. In matched control adoptees, of biological parents free from psychiatric illness, we found less psychiatric illness but an equal number of stressful events during childhood when compared to index adoptees. These results are consistent with a stress-diathesis model, where both genotype and environment contribute to adult illness.

Further analyses of these data revealed that the most common stressors among index subjects with schizophrenia or SPD were troubled family environments. These results are consistent with the current body of literature investigating expressed emotion as a factor in the relapse of adult schizophrenic patients.

3. The Israel Kibbutz--High Risk Study

In 1984, the Laboratory published in the Schizophrenia Bulletin a report of work begun in 1962 on the study of children at risk for schizophrenia in Israel, which was designed and initiated by David Rosenthal. The study has examined 100 children, of whom 50 had one schizophrenic parent (index subjects), and 50 were born to two nonschizophrenic parents (control subjects). Half of both the index and control groups were reared in towns in traditional nuclear families, while the remaining half were reared in communal settings on kibbutzim.

In broad outline, the results indicate that index children were discriminable from controls in many areas of function, but kibbutz and town children did not differ on the experimental examinations. Furthermore, kibbutz versus town rearing had no discernible effect on the performance or behavior of the high-risk index children. Index children were found to be poorer in psychosocial adjustment, perform more poorly in school, manifest a number of neurological "soft signs," and show deficits on psychological tests requiring high levels of attention, visual integration, and visuomotor coordination. An important negative finding was lack of differences between index and control children on psychophysiological measures of arousal and

habituation in the first examination.

We have also conducted follow-up interviews with the study subjects, who are now in their mid-twenties, at the peak of their risk period for schizophrenic breakdown. Ninety of the surviving 99 subjects have been seen. Results show that nine subjects fall within the "schizophrenia spectrum" (of whom six have DSM-III schizophrenia), six from kibbutz backgrounds, and three from towns. When all DSM disorders are considered, more than five times as many ill subjects fall within the index (N=23) than within the control group (N=4). Furthermore, when schizophrenia itself is excluded, the remaining subjects with history of illness (including DSM-III Major Affective Disorder or Dysthymic Disorder) are found predominantly in the index-kibbutz cell (16 of the total of 23 in the cell, including 9 with affective disorder). Other significant preliminary results include persistence of attention-related deficits in the index group, and continued poor social and work adjustment in high-risk subjects.

During the past year we have initiated a second follow-up of these subjects, who are now in their early thirties. At this time, the majority of the subjects who will develop a schizophrenic disorder should have become ill. In order to diagnose these subjects accurately, a Hebrew version of the SADS-L with modifications allowing the accurate assessment of schizophrenia spectrum disorders, has been developed. Social workers who are blind to the subjects' index or control status have been trained in its use. In addition, our laboratory has developed and validated Hebrew versions of neuropsychological tests for use with this population. Subjects have been contacted and are currently being interviewed. As of this writing, we estimate that 40 of the subject cohort have been seen.

Significance to Biomedical Research and to the Program of the Institute

The issue of the mode of heritability of schizophrenia, and factors which modify its development, may be the highest priority of the Institute. This work contributes significantly to our knowledge in this area and ultimately, to our capacity to treat and prevent schizophrenia and related disorders. Our study of childhood stress and adult schizophrenia spectrum disorder suggests a possible direction for prevention of adult psychiatric morbidity.

The studies here, which focus on schizophrenia spectrum disorders as well as pure DSM-III schizophrenia, aid in the identification of milder syndromes genetically linked to schizophrenia. The identification of such syndromes would aid in the search for clear pedigrees of schizophrenic illness by allowing more individuals to be studied and tested for biological markers than the current low number of biological relatives with frank schizophrenia.

Proposed Course

Genains: Drs. Quinn and Mirsky maintain contact with the Genains and exchange correspondence on an occasional basis. We are considering the possibility of inviting the Genains back to NIMH for another series of follow-up studies to include electrophysiological and other measurements that

were not obtained during their visit in 1981.

Denmark: We are investigating the possibility of a follow-up study of the Danish adoptees first studied by Rosenthal. At the time of their previous assessment, a portion of the subjects was too young to have passed through the major risk period for schizophrenia. Such a study would allow for continuing investigation of the longitudinal course of schizophrenic illness as well as potentially confirmatory evidence on the role of stress in the development and relapse in schizophrenia.

Israel: In addition to the current follow-up study of this population, our laboratory has also taken steps to validate previously reported findings from this unique sample. We are planning to interview the siblings of the probands in this study in order to increase economically the sample size and the power of statistical tests conducted with this sample. In addition, we are planning to reinterview the parents in order to use contemporary diagnostic tools to confirm the earlier diagnosis of schizophrenia in index subjects' parents. In addition, family history interviews of these families will help clarify the role of familial schizophrenic and affective illness in the development of psychopathology among these subjects.

As the interviews are completed, we will be collecting neuropsychological test data as well as CT images from consenting subjects. This information will also build on earlier studies of this population to present a fuller picture of the psychobiological and environmental factors leading towards adult psychiatric illness.

Expressed emotion: Our current findings in the Danish sample, taken in conjunction with the developing literature on expressed emotion, suggest that further exploration of the social environment of adult schizophrenic patients may be useful in identifying modifiable environmental factors that could reduce the incidence of relapse. We are currently developing an approach to the study of Expressed Emotion that would shift the focus away from the parents of schizophrenic adults living at home and place it on the social environment of the larger number of schizophrenic patients who live outside of their family of origin. We believe that this approach will lessen the potential for inappropriate stigmatizing of the families of schizophrenic patients, and lead to more effective support strategies for adult schizophrenic patients.

Publications

Mirsky, A.F., Quinn, O.W., DeLisi, L., Schwerdt, P. and Buchsbaum, M.: The Genain quadruplets: A 25 year follow-up of four monozygous women discordant for the severity of schizophrenic illness. Cohen, G. and Miller, N.E. (Eds.): Schizophrenia, Paranoia, and Schizophreniform Disorders in Later Life, in press, 1987.

Mirsky, A.F., Duncan, C.C., Silberman, E.K., Nagler, S., Kugelmass, S., Sohlberg, S., and Shotten, J.H.: Early neuropsychological and other behavioral predictors of later psychotic disorder. In Shagass, C., Simpson,

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- Mirsky, A.F.: The Frontal Lobes. In Stuss, D.T., and Benson, D.F. (book review): J. Clin. Neurophysiol. in press, 1987.
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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00484-27 I.P.P.

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychophysiological Responsivity and Behavior in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Theodore P. Zahn, Ph.D. Research Psychologist LPP, NIMH

COOPERATING UNITS (if any)

Laboratory of Socio-Environmental Studies, Child Psychiatry Branch, Laboratory of Clinical Science, Neuroscience Branch, Biological Psychiatry Branch, and Clinical Neurogenetics Branch, NIMH; Hypertension-Endocrine Branch, NHI.BI.

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

0.8

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The general purpose of this project is to investigate the roles of autonomic nervous system (ANS) activity, attention, and information processing and their interrelationships in the pathology, etiology, and prognosis of psychiatric disorders. A second purpose is to determine biological and psychological processes related to ANS activity and attention. ANS activity is assessed by peripheral measures, such as skin conductance, heart rate, and skin temperature. Subjects are tested under conditions of rest, presentation of tones, and performance on tasks such as reaction time and mental arithmetic.

Biological mechanisms are investigated by correlating these variables with enzyme activity, neuropeptides, and levels of biogenic amines and their metabolites and with brain dysfunction as revealed by CT and PET scans.

Studies are being done on unmedicated patients with diagnoses of schizophrenia, affective disorder, obsessive compulsive disorder, anxiety-panic disorder, and autism to test the diagnostic specificity of patterns of ANS activity. Children of parents with bipolar affective disorder are being studied to determine a possible ANS trait markers. In some studies blood samples are taken during ANS recording sessions in which stressful procedures are given. In one, the effects of success and failure to escape an aversive noise are assessed, and in another, the effects of a dose of yohimbine is being studied. Clinical trials of various treatments are studied in various groups.

Psychological correlates are studied via clinical background data, clinical ratings and questionnaires, and by procedural variations. The use of confirmatory factor analysis in data reduction and to improve quantification of ANS activity is being explored.

Project DescriptionA. Other Personnel

Allan F. Mirsky, Ph.D.	Chief	LPP, NIMH
Carmi Schooler, Ph.D.	Acting Chief	LSES, NIMH
Dennis Murphy, M.D.	Chief	LCS, NIMH
David Pickar, M.D.	Chief	SCS, NSB, NIMH
Thomas Uhde, M.D.	Staff Psychiatrist	BPB, NIMH
Judith Rumsey, Ph.D.	Staff Fellow	CHP, NIMH
Judith Rapoport, M.D.	Chief	CHP, NIMH
Frank Putnam, M.D.	Staff Psychiatrist	LDP, NIMH
John Nurnberger, M.D.	Medical Officer	CNG, NIMH
Alan Breier, M.D.	Clinical Associate	NSB, NIMH
Joseph Zohar, M.D.	Visiting Associate	LCS, NIMH

B. Objectives

The major objective of this project is the further understanding of the role of autonomic nervous system (ANS) activity, information processing and attention, and their interrelationships in psychiatric disorders, primarily schizophrenia. The overall strategy involves studies of ANS and attentional relationships to diagnosis and prognosis, studies of the effects of drugs and other therapeutic interventions, "high risk" and personality studies in normal volunteers, studies of the effects of various types of stress, and studies of the measurement of ANS activity.

C. Methods Employed

The general methods of these studies include measurement of ANS activity through skin conductance (SC) usually measured bilaterally, heart rate (HR), vascular activity (skin temperature and finger pulse volume), and respiration while subjects are resting, exposed to a series of nonsignal tones of constant or variable intensity, and performing tasks. Tasks include tests of attention using reaction time techniques, tests of perceptual speed, and tasks designed to be moderately stressful. A mini-computer system is used to run the experiments and to collect and analyze the data. Studies in various stages of completion are listed below.

1. Schizophrenia Studies

a. A study of newly admitted, drug-free patients used three tasks varying in stressfulness and task demands to test the hypothesis, developed in previous studies, that schizophrenics' ANS does not respond appropriately to variations in stimulus significance. This study also includes several rest periods and a series of nonsignal tones for comparative purposes.

b. In current studies, ANS recording is being carried out in two sessions which include rest periods, a tone series, and two reaction time tasks. In addition, several methods of assessing more precisely the nature of

schizophrenic attention deficits using reaction time (RT) techniques are being compared. (See Z01 MH 00484-25 LPP, 1984-85 for details.) Patients are being tested on standard neuroleptic medication as well as drug free. The purpose of this is to compare our results to most of the recent published non-NIH studies which, for the most part, use medicated patients. Some patients are also being tested in the "learned helplessness" paradigm described in section 3b below.

c. Tests of the ANS effects of drugs such as pimozide, lithium, naloxone, GHB, verapamil, propranolol, and prazosin, and treatments such as hemodialysis and plasmapheresis, have been carried out.

2. Studies on Nonschizophrenic Psychopathology

a. Several confirmed psychophysiological "markers" of schizophrenic pathology have been detailed in previous annual reports and are summarized below. In order to determine which of these are specific to schizophrenia, patients with other types of psychopathology are being tested on the initial part of the current standard protocol (described in 1.b. above) after being medication free for an appropriate time. These include patients with major depressive, obsessive-compulsive, and panic-anxiety disorders (see Z01 MH 00071 BP, 02184 NS, 00153 CHP, and 00336 LCS). In addition, a group of young men who had a diagnosis of early infantile autism have been studied (see Z01 MH 00178 CHP). Some patients with affective disorder and premenstrual syndrome are being tested in the "learned helplessness" paradigm described in section 3b below.

b. Drug effects are being evaluated in several groups. Some panic-anxiety patients are tested on imipramine (double-blind) and will be compared with patients given placebo, and some we are able to test under both treatments. We have completed a study of the psychophysiological and attentional effects of a challenge with yohimbine--a noradrenergic alpha-2 antagonist--using a protocol containing rest periods, a mental arithmetic stress, and a continuous performance task. This was given before and after placebo and yohimbine on two separate days in a double-blind crossover design. Fifteen patients with panic disorder, seven of whom are on alprazolam treatment, are compared with 12 normal controls. This study is a collaboration with Drs. Albus of NSB and Uhde of BPP.

c. Some studies are assessing state changes independently of pharmacological treatment. Earlier annual reports described studies of state changes in multiple personality patients. In collaboration with CHP we are doing follow-up studies on formerly adolescent patients with obsessive-compulsive disorder. Some of these patients should be basically free of symptoms, allowing us to separate out "state vs. trait" influences.

d. Nine patients with presumed early stage Alzheimer's disorder have been compared with 6 controls of about the same age with the aim of discerning if aspects of memory such as those involved in habituation of physiological responses and sequence effects in reaction time studies show deficits

comparable to episodic memory deficits.

e. Patients with high levels of plasma norepinephrine (NE) were compared with patients with similar diagnoses who have low NE in collaboration with LCS and NHLBI. This study should help clarify the role of NE in psychophysiological activity.

f. As part of a larger LPP project, we are testing subjects with known brain lesions from head injuries on the standard ANS and attention protocol used with schizophrenics. The purpose is to determine what specific brain areas may be involved in schizophrenic psychopathology.

3. Studies on Normals

a. In collaboration with CNG, we are testing the offspring of patients with bipolar affective illness to determine if some of the putative ANS trait markers for affective disorder reported in the literature can be considered to be genetic markers.

b. A collaborative study with NSB is designed to compare temporary states of "learned helplessness" and active coping on learning, mood, ANS activity, plasma catecholamines, and cortisol. Subjects are given the task of learning how to turn off an aversive noise. In one condition (nonescape), the problem is insoluble and in the other (escape), it can be solved by correctly pushing a button. The object is to study the neurobiology of a temporary model depressed state in humans.

c. A study on 95 normal subjects tested the hypothesis that ANS activity mediates the relationship between platelet MAO activity and the personality trait of sensation seeking. This study in collaboration with LSES (see Z01 MH 00674) also uses a method of confirmatory factor analysis to reduce psychophysiological data as described in previous annual reports.

4. Major Findings

a. Schizophrenic Studies

1. Confirmation of previous findings of high autonomic base level and a sluggish response to the mild stress of task performance in schizophrenics was observed and was more extreme in eight patients with significant cortical atrophy as shown by CT scan. These and other findings have been detailed in previous annual reports.

b. Data are still being collected. The new data continue to show that the phenomena of visual sensory dominance and intersensory facilitation found in normal subjects also occur in schizophrenics. A subsample of these patients have been used as a comparison group for the autism study described below.

2. Studies on Nonschizophrenic Psychopathology

a. Results for obsessives and autistics were presented in some detail in previous annual reports. Both groups showed distinct patterns of ANS activity across the various conditions which differed from their respective control groups and from schizophrenics. Obsessives showed generally elevated ANS activity like schizophrenics, but unlike them had normal responses to stimuli and tasks. Autistics had very rapid breathing, but normal or only slightly elevated ANS base levels, showing a different pattern from schizophrenics. However, they had even more markedly attenuated ANS responses to significant stimuli and situations than schizophrenics and a unique laterality of SC responses. The data suggest dysregulation of ANS activity by brainstem mechanisms and a problem in mobilizing cognitive processing resources in autism. A paper on autism has been published.

We have retested 19 of the obsessive child group and 20 controls after a three year period. Preliminary results show higher heart rates in the patients at followup but only nonsignificant differences in SC variables. The group x sex effects observed on the original testing were no longer significant at followup. However, a comparison of baseline and followup data revealed that controls decreased significantly more on spontaneous SC fluctuation rate and heart rate than the patients. The state vs trait hypothesis could not really be tested because most patients were still at least moderately symptomatic. Neither the followup psychophysiology nor the change from baseline were significantly related to current clinical state. However, the number of SC orienting responses on the original test correlated with severity of followup symptomatology, and a similar trend occurred for heart rate. Nonsignificant trends in the same direction were present in the other two SC arousal indicators. Thus it may be that obsessive adolescents with a greater biological predisposition are more refractory to long term remission than patients without this trait. Data for other groups are still being analyzed.

b. Results for the yohimbine challenge were described more fully last year. Briefly, yohimbine produced differential effects compared to placebo on mood and heart rate in patients with panic disorder than in controls irrespective of whether the patients were being treated with alprazolam. Heart rate variability and serum cortisol increased about the same amount in both groups and SC activity was not greatly affected by the drug in either group. The data from this study support the hypothesis of alpha-2 hypersensitivity in panic disorder and suggest that hyperventilation may contribute to the genesis of panic attacks. We are awaiting analyses of plasma catecholamine data before writing up these results.

c. The multiple personality project results were reported previously and will be prepared for publication.

d. In preliminary analyses, the Alzheimers patients showed generally a strong trend for hyporesponsivity in skin conductance but because of one or two outliers in each group, these results are not significant. None of the

patients failed to exhibit orienting response habituation, so no evidence of a short term memory deficit at that level was apparent. The only significant psychophysiological group difference found so far is smaller heart rate variability in the patients, suggesting a cholinergic deficit. The low SC activity could be attributable to this mechanism as well. More subjects will be needed to confirm the group trends. Correlations of the individual differences in ANS activity with behavioral, neuropsychological and brain imaging data will be done.

e. Only six subjects with low resting plasma norepinephrine (NE) and five subjects with high NE were available for testing. Aside from a trend ($p = .07$) for a higher resting HR in the high NE group the physiological results were essentially negative. This confirms our previous findings using a within-subject design that changes in plasma NE are related to changes in HR but not in other aspects of ANS activity.

3. Studies on Normals

a. We have done preliminary analyses on data from 22 subjects at genetic risk for affective disorder (Risk group) and 27 controls, 15 to 25 years old. Since there is some evidence in the literature of low SC activity in endogenous depression this channel was examined carefully. The results show no significant differences in SC levels under any condition. The SC orienting response showed nonsignificant differences opposite to the expected direction: More controls (14%) than high risk subjects (9%) were nonresponders, while 40% of the Risk group vs. 25% of controls failed to habituate. There were no differences in SC response amplitudes to innocuous tones. Significant electrodermal hyperresponsivity was seen in the Risk subjects in their response to the more stressful aspects of the protocol--the instruction and practice period for a reaction time task and during a mental arithmetic task. In addition, there were significant differences in SC laterality: The Risk group had larger SC responses to significant stimuli on the left hand and controls on the right hand. Similar differences in affectively ill patients have been reported. These results suggest that low SC activity is not a likely genetic marker for bipolar affective disorder. Rather, persons at risk show ANS hyperresponsivity to mild stress and lateralized information processing differences from controls. The mood scale showed highly significantly greater ratings of depression during the stressful part of the session in the Risk group, compared to controls but no differences in anxiety or aggression ratings.

b. Preliminary results for the first 10 subjects in the learned helplessness study were reported last year. The major findings were greater elevations of ACTH, electrodermal activity and dysphoric mood following the nonescape procedure. A paper on these subjects has been submitted for publication. More extensive data analysis on these and more controls, 18 patients with diagnoses of affective disorder and several patients with other diagnoses is in progress.

c. The major results were reported previously and have now been

published.

Significance to Biomedical Research and the Program of the Institute

Investigations of ANS activity and attention in psychiatric disorders, especially schizophrenia, have produced promising results which suggest that these processes may play fundamental roles in the etiology and expression of the disorders. Limitations on inferences to be drawn from measures of ANS activity come from incomplete understanding of their biological and psychological determinants. One of the main goals of this research is to increase this understanding by investigations of biological and psychological correlates and improving measurement techniques. The dynamic nature of these measures permits the study of processes such as adaptation, habituation, response to and recovery from stress, and effects of single stimuli through noninvasive techniques. Thus, further understanding of their mechanisms could greatly increase their utility in investigations of psychopathology. Continued investigations of the diagnostic specificity of these processes and of their relationships to other clinical features and to prognosis are necessary to confirm and extend our previous results and to test the limits of their generality.

Proposed Course

Analysis of data will continue for the completed project on schizophrenia with the goals of determining the relationship of ANS variables to diagnosis, task performance; and a number of clinical and biological variables available on these patients. Concept modeling by confirmatory factor analysis may be extended to this group.

Collection and analysis of data will continue for current projects on schizophrenic and nonschizophrenic psychopathology and in normal controls. We hope to have sufficient data this year to make a formal test of the diagnostic specificity of ANS activity.

We are starting to test obsessive children on pharmacological treatments in order to determine if ANS effects of the treatments are related to their clinical effectiveness. This will attempt to replicate and extend a previous similar study with adult obsessives from our laboratory.

Tests on children in the Hopkins preventive intervention project whom LPP is negotiating to bring in. We will use the same test protocol as we used previously with hyperactive, obsessive, and anxious children, and currently with children having a diagnosis of conduct disorder (see Z01 MH 00486 LPP). This study should determine how ANS activity relates to the extensive classroom behavior observations on these children and how these and other observations may complement each other in predicting future psychopathology or behavior problems.

We are planning to design a new protocol for schizophrenics by which to test the hypothesis that schizophrenics and controls exhibit differential

effects of increases in arousal on attention. Arousal will be manipulated by changes in posture and variations in stimulus intensity and significance. We also plan to obtain measures of smooth pursuit eye tracking on schizophrenic patients, since Dr. Hommer of NSB, who had been doing this has left the program. There is evidence that smooth pursuit eye movement dysfunction may be a genetic trait linked to schizophrenia, so it is important to obtain this measure on these patients.

Publications

Goldstein, D. S., Bonner, R. F., Zimlichman, R., Zahn, T. P., Cannon, R. O., III, Rosing, D. R., Stull, R., and Keiser, H. R.: Indices of sympathetic vascular innervation in sympathectomized patients. J. Auton. Nerv. Syst., 15: 309-318, 1986.

Zahn, T.P., Schooler, C., and Murphy, D.L.: Autonomic correlates of sensation seeking and monoamine oxidase activity: Using confirmatory factor analysis on psychophysiological data. Psychophysiology, 23: 521-531, 1986.

Zahn, T. P., Rumsey, J. M., and Van Kammen, D. P.: Autonomic nervous system activity in autistic, schizophrenic, and normal men: Effects of stimulus significance. J. Abnorm. Psychol., 96: 135-144, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00486-15 LPP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychophysiological Effects of Stimulant Drugs in Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Theodore P. Zahn, Ph.D. Research Psychologist LPP, DIRP, NIMH
Other: Judith Rapoport, M.D. Chief CHP, NIMH
Martine Flament, M.D. Guest Researcher CHP, NIMH
Marcus Kruesi, M.D. Clinical Associate CHP, NIMH

COOPERATING UNITS (if any)

Child Psychiatry Branch, NIMH

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH/ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.3

PROFESSIONAL:

0.2

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Tests of the effects of acute and chronic administration of caffeine on autonomic nervous system (ANS) functioning have been carried out to evaluate the role of ANS activity in the behavioral and subjective effects of this drug. A test of attention using a reaction time method is included.

The test protocol involves recording peripheral indicators of ANS activity such as skin conductance (SC), heart rate (HR), and skin temperature during a session consisting of a rest period, presentation of a series of simple tones to which no response is required, and the reaction time task. Studies have been carried out on the effects of the acute administration of two doses of caffeine and a placebo in 6-13 year old boys and in men, and a study of chronic (2 week) caffeine intake in children.

The effects of both acute and chronic administration of caffeine were increases in SC indices of arousal but some trends toward decreases in HR. The SC results are consistent with the hypothesis that caffeine can be considered a pharmacologic model for anxiety, but the HR effects suggest the model is imperfect.

The most recent study, an acute dose protocol with caffeine was conducted on children with anxiety disorders and controls. This tested the hypothesis, for which there is evidence in adults, that patients with anxiety disorders are more sensitive to caffeine than controls.

Another current study compares ANS activity and attention in boys with diagnoses of Conduct Disorder and Attention Deficit Disorder.

Project Description

This project has evolved from the study of hyperactivity in children (now called Attention Deficit Disorder) to the study of stimulant drugs--dextroamphetamine and caffeine--in children and adults.

Results of the caffeine studies with normal subjects have been presented in previous annual reports. The acute dosage studies were published this year. The pattern of ANS results for caffeine was different from that of another "stimulant drug"--dextroamphetamine--in that caffeine produced very consistent and strong increases on SC activity but minimal or opposite effects in HR, while amphetamine dramatically increased HR and had less consistent effects on SC activity (although it also generally increased it).

The recent study of anxiety disorders, mentioned above, used the same protocol as in some of our acute studies. Children were tested at baseline, then in three sessions where they receive, randomly, 0, 3, and 10 mg/kg of caffeine one hour before testing. Children with a diagnosis of anxiety disorder and normal controls have been tested. Results on eight patients and eight controls show that, as in our previous studies, caffeine increased SC activity and decreased HR. Contrary to expectation, caffeine effects on SC activity were not greater in the anxious children. On the contrary, caffeine produced more electrodermal activation in the controls, significantly so in some cases. These effects could not be accounted for by a difference in placebo values as these were not generally much different. In contrast, the anxious subjects showed a somewhat greater decrease in heart rate than controls, but this might be due in part to higher heart rate on placebo in the anxiety group. Side effects were not different in the two groups (see Z01 MH 00161-08 CHP).

Testing of boys with diagnoses of conduct disorder (CD) and attention deficit disorder (ADD) is being done with two general objectives in mind. One is to look for ANS markers of diagnosis. Previous research in this laboratory and others has shown that ADD boys do not differ from normals in indices of arousal but have generally lower ANS responsivity to stimuli. The literature on psychopathic personality in adults--a possible outcome of CD--shows evidence of low SC levels and diminished SC (but not HR) reactivity to nonsignal stimuli. Our ANS protocol will allow tests of these differences in the boys. A second objective is to explicate the attention deficit in ADD children and to test whether CD boys have similar deficits. A battery of simple and choice reaction time tasks, similar to those given to schizophrenics are being used for this purpose (see Z01 MH 00484-25 LPP, 1984-85).

Significance to Biomedical Research and the Program of the Institute

The ANS effects of caffeine consistently found in these studies partially resemble those seen in anxiety states and other psychopathology. Since caffeine effects are thought to be mediated by blockade of adenosine receptors, these studies may help determine the mechanisms involved in those

aspects of ANS activity that are components of anxiety states. Our finding that children with anxiety disorders are not especially sensitive to caffeine effects is surprising in terms of the usual models of caffeine effects in adults with anxiety disorders and suggests either that these models do not apply to children or that they generally need revision.

The study on CD and ADD boys may also help determine the mechanisms of ANS measures through correlation with the extensive neurobiological data being obtained in this group. It should also provide evidence of a possible biological basis for the diagnostic distinctions. This study is relevant to a major objective of LPP to develop a taxonomy of attention disorders.

Proposed Course

Continued collection of data on the CD-ADD project is planned. The analysis of the data will include correlation of the ANS and attention data with metabolites of biogenic amines from CSF.

Publications

Zahn, T.P. and Rapoport, J.L.: Acute autonomic nervous system effects of caffeine in prepubertal boys. Psychopharmacology, 91: 40-44, 1987.

Zahn, T.P. and Rapoport, J.L.: Autonomic nervous system effects of acute doses of caffeine in caffeine users and abstainers. Int. J. Psychophysiology, 5: 33-41, 1987.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00491-1J I.P.P.

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Personality Factors and Psychophysiological Responses to Changing Stimulus Input

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Theodore P. Zahn, Ph.D. Research Psychologist LPP, NIMH

Other: Thomas N. Robinson, Jr. Guest Researcher LPP, NIMH

COOPERATING UNITS (if any)

NIH Normal Volunteer Office.

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.50

PROFESSIONAL:

0.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are to investigate relationships among differences in personality, sensory thresholds, and autonomic nervous system (ANS) activity in normal humans and to study racial differences in ANS activity. Bilateral skin conductance and heart rate have been recorded in two sessions in which constant and variable intensity tones and lights are presented and auditory and two-flash thresholds (TFT) determined by methods which permit signal detection analyses. A procedure for determining the uncomfortable loudness level has also been used. Several standardized personality tests were also given. These include scales of sensation-seeking, extraversion, neuroticism, psychoticism, field dependence and anxiety. In addition comprehensive measures of lateral dominance have been given as well as a measure of "torque" (clockwise drawing of circles) which has been hypothesized to reflect a neurointegrative deficit and be related to risk for future psychopathology. A procedure for manipulating ANS arousal experimentally with minimal distracting effects--a change in posture from supine to standing--is being used to study such problems as the effects of base levels on responsivity, the effects of arousal on performance, and the effects of personality variables on this relationship. This project allows testing of several theoretical models of the relationships of ANS activity, sensory sensitivity, and personality, some of which have implications for the etiology of psychopathology. Tests of the relationships between laterality in skin conductance variables and behavioral laterality will also be done to see if inferences about lateralized brain function can be made from such variables.

Project Description

A. Objectives

A large body of psychological literature postulates that an important dimension of individual differences in behavior or personality is reflected in the reactions of the nervous system to sensory stimulation. Pavlov's original conception of "strong" and "weak" nervous types has been modified and extended by Western theorists to reflect such personality dimensions as "extraversion-introversion," "sensation-seeking," and "field dependence," each of which can be measured by a questionnaire or other test procedures. The theoretical models that have been built up from these concepts have implications for interrelationships among personality, autonomic nervous system (ANS) base levels and responsivity to stimulation, and sensory sensitivity. There are also implications for psychopathology, in that schizophrenics have been considered to be extremely "weak" nervous types in the Pavlovian system (i.e., overreactive to weak stimulation and underreactive to strong stimulation--"transmarginal inhibition"). Another development is the more recent delineation by H. Eysenck of the dimension of "psychoticism."

The major objective of this project is to test some of the implications of these models of personality by interrelating the personality measures with sensory thresholds and sensitivity, and ANS activity in normal humans. Other objectives are to assess racial differences in ANS activity and in its relationships to the other variables in the study and to explore relationships of differences in the laterality of skin conductance activity with behavioral assessments of laterality, and to test the effects on ANS activity increasing arousal by means of a postural change.

B. Methods Employed

Over 200 normal volunteers have been assessed on several personality dimensions, including the three Eysenck scales of extraversion, neuroticism, and psychoticism in addition to, field dependence, sensation-seeking, impulsivity, ego strength, and anxiety, assessed for degree of lateral dominance, and given tests of ANS and sensory functioning in the various protocols described earlier. Since not all subjects have received all procedures, the results presented below are based on partially overlapping subsets of subjects for different comparisons.

In another protocol a fixed foreperiod reaction time procedure is included similar to that used with patients in Z01 MH 00484 LPP.

C. Major Findings

In previous annual reports, relationships between questionnaire-defined personality variables, ANS activity, and sensory thresholds have been described. In general, subjects with high scores on the Eysenck personality scales of extraversion, psychoticism, and, surprisingly, neuroticism tend to

have low ANS activity and reactivity. Low ANS activity was also found in subjects with high scores on a scale of schizotypal personality. Subjects high on sensation-seeking were also very responsive autonomically to novel stimuli. Low sensory sensitivity was shown by subjects high on psychoticism and those showing a "torque" (clockwise) pattern of drawing a circle.

Recent analyses of this large data set have attempted to test the utility of the concept of strength of the nervous system to unify personality, sensory sensitivity, and physiological data. According to the theory, subjects with a "weak" nervous system type should be high on sensory sensitivity and have a low threshold for aversiveness as stimulus intensity increases, have high ANS activity, and be low on sensation seeking, extraversion, and ego strength, and high on anxiety and field dependence.

Results show that there were many confirmatory results for the model, many neutral results, and few disconfirmatory ones, such that some predictions from the model fared better than others. The ego strength measure was related to all three sensory measures in the predicted direction but not to the physiological data, while sensation seeking, extraversion, and field dependence showed some expected relationships with the ANS data but not to auditory sensitivity or aversiveness. Sensation seeking and intraversion were predictive of a low two-flash threshold, however. ANS activity was related to auditory sensitivity and TPT but not to aversive level. Thus the general pattern of the results seems to fit the model reasonably well, but there are some inconsistencies in the individual measures. Possibly there is more than one dimension in the strength construct.

A preliminary analysis of the reaction time data in the latest protocol shows that introverts had slower reaction time than extraverts and showed more slowing as the length of the preparatory interval increased. This is similar to the usual finding in schizophrenia.

Significance to Biomedical Research and the Program of the Institute

The construct of the strength of the nervous system, whatever it may be thought to denote in terms of neuronal functioning, has a traditional relevance to psychopathology in that a weak nervous system is said to be characteristic of schizophrenia. The association with the Western measures of introversion and low ego strength is also relevant in this context, as are the associated phenomena of enhanced sensory sensitivity and elevated ANS activity. Although there are wide subtype differences among schizophrenics, the concept of a schizophrenic characterized by social withdrawal, weak ego boundaries, and high arousal who is overwhelmed by environmental stimuli has been associated with the early stages of the illness. The data from this project appear to support the existence of a similar syndrome in normal subjects, albeit to a lesser degree. Thus this approach may be considered as an alternative to the increasingly popular "high risk" study using scales designed to measure some form of schizotypy by assessing symptom-like phenomena more directly.

Increased understanding of the relationships among autonomic, perceptual, and personality variables in normal subjects should be of great assistance in interpreting the autonomic and perceptual results from studies on psychopathology in which similar methods are used. This project has been very useful in the development of protocols for studies of psychopathology.

Proposed Course

A priority is to attempt to delineate further the strength of the nervous system construct. To this end multivariate analyses such as multiple regression and/or confirmatory factor analysis will be applied to this large data set. We also plan to do more data collection and analysis with the newer reaction time protocol.

We have some as yet unanalyzed psychophysiological data collected in two "high risk" studies in which extremely poor or good performance on either the Continuous Performance Task or pendulum eye tracking (both of which are impaired in schizophrenia) were selection variables. These data are obviously quite relevant to the questions discussed here and we plan to analyze them.

We are planning also to develop a new battery of tests that vary in their sensitivity to arousal, making use of some of the recent developments in the field of cognitive psychology.

Publications

None.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00503-07 LPP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Human Clinical Studies of Attention Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Allan F. Mirsky Chief LPP, NIMH

COOPERATING UNITS (if any)

Epilepsy Branch, NINCDS; Clinical Neurosciences Branch, NINCDS; Laboratory of Clinical Sciences, NIMH; Neuropsychiatry Branch, NIMH; Boston University

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.25

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research comprises three related areas of investigation concerned with specifying neuropsychological factors underlying clinical conditions in humans in which disturbed attention is a major symptom. A major emphasis is on (1) illuminating the nature of brainstem pathophysiology, if any, in such entities as petit mal or absence epilepsy, infantile autism, schizophrenia, and related diseases; (2) an additional major emphasis is on extending the neurobehavioral analysis of attention loss in absence epilepsy so as to facilitate developing alternative treatment strategies for such patients. Both of these projects form part of a larger effort which is aimed at (3) developing a comprehensive and systematic taxonomy of attentional disorders in humans. This latter study will eventually comprise study of patients with cerebral lesions, seizures, dementing diseases, and metabolic illnesses of the brain.

Project DescriptionA. Other Personnel

Connie C. Duncan, Ph.D.	Senior Staff Fellow	LPP, NIMH
Walter H. Kaye, M.D.	Associate Professor	Univ. of Pittsburgh
Richard Coppola, D.Sc.	Senior Engineer Officer	CBD Branch NIMH
Theodore P. Zahn, Ph.D.	Research Psychologist	LPP, NIMH
Roger Porter, M.D.	Chief	EBB, NINCDS
Debbi Fein, Ph.D.	Assistant Professor	Boston Univ.
Daniel R. Weinberger, M.D.	Chief	CBD Branch NIMH

B. Project Description1. Brainstem Mechanisms in Attention Impairment

Current approaches to the neuropsychology of attention impairment have emphasized that the system responsible for the maintenance of attention or consciousness within the brain is most likely represented at a variety of levels of the neuraxis. From an evolutionary point of view, it is clear that the capacity for sustained attentive behavior is present in many species which do not possess more than a rudimentary forebrain or telencephalon. MacLean's analysis of the R-complex within the human brain leads to the view that this "clump of ganglia," which constitutes virtually all of the reptilian brain, can support a variety of ritualistic, repetitive behaviors which could be characterized as sustained and attentive. Evolution progressed and the brain developed additional complexity and volume. Additional capacity for attentive behavior was thus overlaid on the more primitive, although in many aspects thoroughly adequate, brainstem system of the reptile. Therefore, although the system for maintenance of attentive behavior in the human (or higher primate) includes limbic and neocortical components, the brain stem remains a key component and possibly the keystone of the entire system. Authors such as Hughlings Jackson and Penfield and Jasper recognized this in their conceptions, respectively, of "highest-level seizures" and the "centrencephalon." In their theorizing, consciousness was either localized in or regulated by deep brainstem structures. Without reviewing all of the evidence that led to those views of the hierarchical organization of attention and consciousness within the brain, we nevertheless point to the extremely deleterious effects on such capacities of small lesions in the brainstem region of the third and fourth ventricles. In the last ten years, a new technological refinement of evoked-potential methodology has made possible an other-than-theoretical exploration of the role of brainstem structures in certain clinical states. This "far field" or BAER (for brainstem auditory-evoked responses) technique makes it possible to assess the integrity of auditory (and somatosensory) relay nuclei within the brain stem of humans. Although the technique has probably had most utilization in the diagnosis of

demyelinating disease, it has also been used in the study of other neurological and, recently, psychiatric disorders. There may or may not be any specific interest in these sensory systems (auditory, somatosensory) in studying a particular clinical entity (i.e., absence seizures, infantile autism); nevertheless, the possibility of evaluating the functional integrity of certain systems within the brain stem is extraordinarily valuable, and many clinical investigators are using these techniques. We have published work indicating that there are disturbances (prolonged transmission time) in the processing of auditory information in the brain stem in infantile autism and in schizophrenia. We have also shown that in absence seizures (spike-wave activity), both naturally-occurring and experimentally-induced, there may be perturbations of auditory brainstem functioning. Several years ago, we completely revised the hardware and computer software used for analyzing BAERs in our laboratory. The data are now clear, clean, reliable, and repeatable and we have run some control studies of normal subjects using parametric variations of intensity, etc.

BAERs are now routinely gathered on our patient subjects and we hope within the coming year to have publishable quality data from schizophrenic subjects and other clinical populations. We are also planning to gather BAERs in patients with head injuries, as well as possibly recalling some autistic subjects for retesting. BAERs are also included in the alcohol protocol being conducted by Dr. Frances Gabbay, a Guest Researcher from John Hopkins University.

2. Neurobehavioral Studies in Absence Epilepsy

We have for a number of years been studying the absence attack in patients with petit mal/centrencephalic/absence seizures (the terms are more or less interchangeable) as a model state to understand the phenomenon of consciousness/attention. Some of these studies have involved comparing the behavioral capacities of patients suffering from petit mal--as opposed to focal seizure disorders; other studies have involved detailed comparison and contrast between the behavioral and the electroencephalographic symptoms/signs of the disorder. Most recently, these investigations have: (1) used evoked potentials in the visual and auditory modalities as indices of the sensory effects of generalized seizure activity of the symmetrical and synchronous wave and spike variety, and (2) examined changes in the EEG power spectrum prior to WS bursts as prodromal signs which may be used to predict (and ultimately to control) WS bursts. We propose to continue this line of neurobehavioral investigation, using event-related potentials of various types as well as other behavioral and physiological tools, to refine further our understanding of the nature of altered consciousness in absence (petit mal) epilepsy.

A group of approximately eight subjects with absence epilepsy has now been studied with a full battery of tests, including a complete neuropsychological examination and a number of ERP paradigms requiring varying amounts of attention. Untreated cases with absence epilepsy are difficult to find and persons with good medication control of their seizures are reluctant to serve

as subjects. Nevertheless, we now have a sufficiently large group to be able to make some additional contributions to the study of attention in absence epilepsy. Analysis of the neuropsychological data is underway. Preliminary results indicate that although this is a high-functioning group of absence patients, they demonstrate the expected impairment in attention in the interictal period, as assessed by the Continuous Performance Test (CPT). Further, it was found that significantly greater impairment was seen in the auditory version of this task than in the visual version.

Analyses of the ERPs to CPT stimuli revealed impairment in information processing which paralleled the behavioral data. In addition, new insights into the response failures in absence epilepsy were provided by this analysis, which is reported in more detail in protocol Z01 MH 00509-05 LPP.

3. A Taxonomy of Attentional Disorders

The goal of this project is to develop a comprehensive and coherent account of the relation between symptoms of altered or disturbed attention or consciousness as they appear in various clinical entities, the other behavioral and clinical characteristics of the several disorders, and the specific central nervous system damage or disturbance in each disorder. The attentive capacities of the patients are assessed by a number of attention tests including the CPT (continuous performance test), a measure of sustained visual attentive behavior. The ultimate goal will be to describe the precise attentive deficit (as opposed to cognitive losses) and the nature of the neuropathophysiology associated with each of the following clinical entities: cerebral lesions (frontal, parietal, temporal lobe, or brainstem); centrencephalic/absence epilepsy; schizophrenia; infantile autism; dementing diseases (Alzheimer's, Korsakoff's, Huntington's); and metabolic diseases (Phenylketonuria, Uremia, Anorexia Nervosa and related illness).

We will attempt, as well, to relate these changes where possible to standardized measures of mnemonic and other cognitive function, and to autonomic indices of attention, arousal, and habituation. Reasonable amounts of data have now been collected on a number of these populations and the work continues.

During the past year, a theoretical model of the elements of attention has been proposed in a number of publications. This model is based on a factor analysis of the data from nearly 100 subjects. In addition, it incorporates information from neuroanatomical and neurophysiological sources. It suggests that "attention" comprises a series of behavioral components or elements including the capacities to focus, encode, sustain, shift and execute. Further, it is suggested that those elements are best assessed by different groups of neuropsychological tests (which are incorporated in our LPP test battery). Additionally, it is speculated that these behavioral elements are supported by different regions of the central nervous system.

The elements of a attention model, it is hoped, will provide a useful heuristic device for organizing studies and analyzing data, and will

facilitate the development of a taxonomy of attention disorders. The model is discussed further in Z01 MH 00508-05 LPP, which also describes its use in screening for attention disorders in a population of second grade public school children.

Significance to Biomedical Research and to the Program of the Institute

Since attention disturbance is a characteristic of many significant psycho- and neuropathological disorders, it is essential to have a clear empirical and theoretical account of the role and pathophysiological significance of this symptom. Such a theoretical model will aid in understanding the etiology and course of these illnesses and may aid in improving their treatment.

Proposed Course

We have a substantial group of schizophrenic, epileptic, and brain-injured patients tested on our laboratory procedures (i.e., CPT, brainstem auditory-evoked potentials, various tests of cognition and memory, autonomic indices of attention, etc.). We are in the process of preparing the results of these studies for publication.

We have completed an edited book on petit mal epilepsy which is in press at this time.

Publications

Mirsky, A.F., and Ray, C.: Studies in the Neuropsychology of Attention Impairment: Human Symptoms and Animal Models. In Galbraith, G.C., Kietzman, M.L., and Donchin, E. (Eds.): Neurophysiology and Psychophysiology: Experimental and Clinical Applications. Hillsdale, N.J., Lawrence Erlbaum Associates, 1987, in press.

Kaye, W.H., Gwirtsman, H.E., George, D.T., Weiss, S.R., and Jimerson, D.C.: Relationship of mood alterations to bingeing behavior in bulimia. Brit. J. Psychiatry, 1987, in press.

Kaye, W.H., Obarzanek, E., George, D.T., Jimerson, D., C., and Eber, M.H.: Caloric intake for satisfactory maintenance in anorexia nervosa: Non-bulimics require greater caloric intake than bulimics. Amer. J. Clin. Nutrition, 1987, in press.

Kaye, W.H.: Opioid Antagonist Drugs in the Treatment of Anorexia Nervosa. In Garfinkel, P.E., and Gardner, D. (Eds.): The Role of Psychotropic Drug Use for Treating Eating Disorders, 1987, in press.

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Coppola, R.: Topographical Representation of Spike-Wave Activity. In Myslobodsky, M.S. and Mirsky, A.F. (Eds.): Elements of Petit Mal Epilepsy, New York, Peter Lang, 1987, in press.

Mirsky, A.F. and Grady, C.: Toward the Development of Alternative Treatments in Absence Epilepsy. In Myslobodsky, M.S. and Mirsky, A.F. (Eds.): Elements of Petit Mal Epilepsy, New York, Peter Lang, 1987, in press.

Mirsky, A.F.: Behavioral and Psychophysiological Effects of Petit Mal Epilepsy in Light of a Neuropsychologically Based Theory of Attention. In Myslobodsky, M.S. and Mirsky, A.F. (Eds.): Elements of Petit Mal Epilepsy, New York, Peter Lang, 1987, in press.

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Mirsky, A.F. and Duncan, C.C.: An Introduction to Modern Techniques of Clinical Neuropsychology. In Wise, T.N., and Fava, G. (Eds.): Advances in Psychosomatic Medicine. New York, Karger, 1987, pp. 167-184.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00504-07 LPP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Models in the Monkey of Generalized Seizures of the Absence Type

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Allan F. Mirsky, Ph.D. Chief LPP, NIMH

Others: Eva Bakay Pragay, Ph.D. Guest Researcher Vienna

Austria

Richard Nakamura, Ph.D. Guest Researcher

Michael Myslobodsky, M.D., Ph.D. Professor,

Univ. of Tel Aviv

Israel

Richard Coppola, Ph.D.

Engineer

CBD Branch

NIMH

COOPERATING UNITS (if any)

Tel-Aviv University, Israel

LAB/BRANCH

Laboratory of Psychology and Psychopathology

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INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.7

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Generalized seizure activity with the electrographic appearance of absence epilepsy (bilaterally symmetrical and synchronous paroxysmal three-per-second spike and wave discharges) can be elicited in the monkey by a variety of methods. These include electrical stimulation of various locations within the brain, injection of convulsant drugs and other substances, and administration of compounds which may alter normal inhibitory mechanisms within the cell. Model seizure states created in these ways are studied in order to test hypotheses about pathophysiological seizure mechanisms, sensory processing and attentional capacities during absence seizures, effects of spike-wave activity on cellular activity, and effects of techniques or maneuvers which may modify or reduce convulsive activity. Most recently this project has involved the following work: we studied the behavioral and electrographic effects of a GABA-enhancer and surveyed the attention-related cells in the frontal lobes of the monkey. Other studies of "attention" cells in inferior parietal and preoccipital cortex have been completed as well.

Project Description

The putative neurotransmitter GABA (γ -aminobutyric acid) is thought to be involved in the central neural processes of inhibition whose perturbation can result in generalized seizure disorders. Two compounds which are metabolically related to GABA are GBL and GHB. γ -butyrolactone (GBL) and the pharmacologically active product of its hydrolytic cleavage, γ -hydroxybutyrate (GHB), produce several central effects of potential significance for therapy and experimental pathology. Winters and Spooner identified GHB effects as epileptogenic or related to "non-convulsant epilepsy." Other research in rodents and monkeys added to the conviction that GHB causes electroencephalographic and behavioral effects akin to petit mal epilepsy.

We attempted to assess the petit mal-like effects induced by GBL by studying the attention-related performance and EEG responses in monkeys administered a single dose of the drug. All animals had been trained to perform a go/no-go visual attention task similar to the Continuous Performance Test (CPT) used in studying human subjects with petit mal. The criterion performance was 80% correct responses.

While there were individual differences in responding after administration of 125 mg/kg of GBL, a dose of 200-250 mg/kg caused a reliable suppression of responding in all subjects. When the testing began 30 minutes following the drug, animals initially responded rapidly and reliably but soon ceased responding altogether. However, they remain sufficiently alert to groom and react to environmental stimuli. Some occasionally resumed responding for several minutes. Offered water, all animals drank it eagerly.

If tested immediately following administration of the drug (200-250 mg/kg), they were able to perform at 60-70% correct; at about 40 minutes the responding came to a complete halt. Here again, monkeys remained competent perceptually and motorically for some time thereafter.

EEG monitored during the task performance showed a build-up of generalized hypersynchronous activity in some areas when performance deficit was noticeable. A pattern resembling 3 cps wave-spike discharges typical of petit mal was never seen either during this period or at the end of the study. These effects are not typical of either petit mal or petit mal status and may be explained by the development of frank sleep. These effects seem related to the general anesthetic properties of GBL (described by some investigators) rather than to its potential (if any) as a model of petit mal.

Significance to Biomedical Research and to the Program of the Institute

This protocol provides information concerning the nature of the attention-support system in the primate brain, the role of various neurotransmitter substances in consciousness and in generalized seizures and contributes to the current efforts to produce an accurate primate-based model of the pathophysiological processes in absence epilepsy.

Proposed Course

The work described here has been accepted for publication in Behavioral Brain Research. Additional findings from this project will be published in the future. We have recently completed a book, which is currently in press, that includes chapters reviewing the recent developments in the biochemistry, electrophysiology and genetics of absence epilepsy. The book will incorporate much of the material germane to this project.

Publications

Mirsky, A.F., and Pragay, E.B.: Brainstem Mechanisms in the Processing of Sensory Information: Clinical Symptoms, Animal Models, and Unit Analysis. In Sheer, D.E. and Pribram, K.H. (Eds.): Attention: Cognitive, Brain Function and Clinical Applications. 1987. In press.

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Myslobodsky, M.S. and Mirsky, A.F.: Theoretical Summary. In Myslobodsky, M.S. and Mirsky, A.F. (Eds.): Elements of Petit Mal Epilepsy. New York, Peter Lang, 1987, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00508-05 LPP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropsychological Evaluation of Psychiatric and Neurological Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Connie C. Duncan, Ph.D. Chief, Unit on Psychophysiology LPP, NIMH

COOPERATING UNITS (if any)

Biological Psychiatry Branch, Laboratory of Clinical Science, NIMH; Developmental and Metabolic Neurology Branch, NINCDS; Chestnut Lodge Hospital; Johns Hopkins University; Maryland Head Injury Foundation; Division of Neuropsychology, Department of Psychiatry, Medical College of Pennsylvania.

LAB/BRANCH

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TOTAL MAN-YEARS:

1.7

PROFESSIONAL:

0.7

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A set of comprehensive neuropsychological test batteries is used to provide a complete assessment of various cognitive and sensory functions that can be related to damage or dysfunction in different regions of the brain. The adult battery comprises tests designed to tap the following aspects of behavior: attention, executive functions, language, memory, motor functions, orientation, selected sensory and perceptual functions, vigilance, and visual-spatial functions. In addition, adults are given a test of general intelligence and a personality inventory. In some studies, subjects are administered a structured psychiatric interview. Modified batteries have been developed for the assessment of infants, preschool children, children ages 5-8, and children ages 6-16. The data provided by these batteries are being used to construct neuropsychological profiles of the neurological and psychiatric diagnostic groups under study in the LPP. The LPP has been particularly interested in disorders involving impaired attention, including schizophrenia, complex partial seizures, eating disorders, affective disorders, and head injuries. Comparisons are being carried out between the neuropsychological profiles of various groups of psychiatric patients and those of patients with known cerebral lesions in specified brain regions. Our data are also being used to delineate neurobehaviorally-defined subgroups within diagnostic categories, an undertaking aimed at reducing variability in psychiatric diagnosis, treatment, and outcome. The data provided by this protocol provide a complete behavioral assessment that may be integrated with concurrently gathered neurophysiological, neuroradiological, and biochemical information.

Project DescriptionA. Other Personnel

Allan F. Mirsky, Ph.D.	Chief	LPP, NIMH
Barbara P. Jones, Ph.D.	Special Expert	LPP, NIMH
Bruno J. Anthony, Ph.D.	Senior Staff Fellow	LPP, NIMH
Emile Brouwers, Ph.D.	Visiting Associate	LPP, NIMH (to 11/1/86)
Mary Beth Ahearn, M.A.	Graduate Associate	Department of Mental Hygiene, Johns Hopkins University
Robert Post, M.D.	Chief	BPB, NIMH
David C. Jimerson, M.D.	Chief	SBP, LCS, NIMH
John Fink, M.D.	Clinical Associate	DMNB, NINCDS
Elkhonon Goldberg, Ph.D.	Director	Division of Neuropsychology, Department of Psychiatry, Medical College of Pennsylvania
C. Wesley Dingman, M.D.	Assistant Clinical Director	Chestnut Lodge Hospital
Sheppard G. Kellam, M.D.	Chairman	Department of Mental Hygiene, Johns Hopkins University
William W. Eaton, Ph.D.	Associate Professor	Department of Mental Hygiene, Johns Hopkins University

B. Objectives

This project has as its goal the investigation of neurobehavioral functioning in neuropsychiatric patients, such that: (1) Neuropsychological profiles of diagnostically distinct groups can be obtained, and differences in the profiles among groups can be used as an indication of specific organic influences in the psychopathology of these patient groups; (2) Neuropsychological profiles of patients within a heterogeneous diagnostic classification can be obtained. Differences between patients can provide neurobehaviorally-defined subgroups that might reduce variability in diagnosis and treatment, as well as improve outcome; and (3) The obtained comprehensive neurobehavioral data can be correlated with electrophysiological, biochemical, and neuroradiological data that are being collected concurrently on these patients to provide a link between pathophysiology and behavior.

C. Methods Employed

The standard adult neuropsychological battery, used for many of our studies, is presented below. Cognitive and sensory functions are presented in tabular form along with the test(s) used to assess them. Modified batteries have been assembled for some of our studies, and we have assembled special

batteries for the assessment of infants and children in three different age groups from 2 to 16. Administration of the adult battery takes 8-12 hours and occurs over several days. Administration of the infant and child batteries takes from 1/2 hour to 6 hours, depending on the age of the child, and is divided into as many sessions as needed to avoid overtaxing the subject.

LPP Adult Neuropsychological Test Battery

FUNCTION

TEST

Executive

Sequencing, Attention
Attention

Perception and Reasoning

Concept Formation and Abstraction

Trail Making Test
Stroop Test
Letter Cancellation Test
Raven Standard Progressive
Matrices
Wisconsin Card Sorting Test;
Category Test (Halstead)

General Intelligence

Wechsler Adult Intelligence Scale-
Revised

Language

Initiation

Lexical
Written

Comprehension

Auditory Discrimination

Controlled Oral Word Association
Test
Boston Naming Test
Boston Diagnostic Aphasia
Examination--Narrative Writing
Token Test (Spreen & Benton)
Semantic Aphasia Test (Goldberg)
Wepman Auditory Discrimination
Test

Memory

Global
Recent Verbal Memory

Recent Visual-Spatial Memory

Remote Memory

Wechsler Memory Scale I
Buschke Selective Reminding Test
Rey Auditory Verbal Learning Test
Recurring Figures Test (Kimura)
Complex Figure Test
(Rey-Osterrieth)
Boston Famous Faces Test (Short
Form)
Television Test
Boston Recall Test (Short Form)

Motor Functions

Purdue Pegboard Test
Boston Apraxia Test

Orientation

Temporal Orientation Test (Benton)

LPP Adult Neuropsychological Battery (continued)

<u>Personality</u>	Minnesota Multiphasic Personality Inventory
<u>Sensory and Perceptual</u>	Bioptr Vision Tests Dvorine Pseudo-isochromatic Plates Titmus Stereo Tests Harris Test of Hand Dominance Eye Dominance Test
<u>Vigilance</u>	Continuous Performance Test
<u>Visual-Spatial</u>	Hooper Visual Organization Test Embedded Figures Test (Witkin) Butters' Embedded Figures Test

D. Major Findings

The test battery or parts thereof has been administered to a total of 302 subjects in our laboratory (107 males, 195 females), of which 70 were control subjects (22 males, 48 females). In addition, 77 follow-up test sessions have been conducted in our laboratory for protocols involving two or more serial neuropsychological evaluations. Outside the laboratory, 485 subjects have been tested in the collaborative study with the Johns Hopkins School of Hygiene and Public Health. The total number of subjects tested, both in and outside of the laboratory and including follow-up testing, is 864.

1. Eating Disorder Patients

Testing of patients with eating disorders and matched normal controls has been continued as a result of the intriguing results of last year's data analyses. To date, 28 underweight anorexic patients (11 restrictors and 17 bulimarexics) have been assessed, and 18 have been retested after short-term weight restoration. In addition, we have tested 40 normal-weight bulimics and 16 long-term weight-restored anorexics (7 former restrictors and 9 former bulimarexics). Reanalysis of the data adding the subjects tested within the past year has, for the most part, confirmed our previous findings. In many cases, the reanalysis has strengthened those findings. First, with regard to psychopathology, we found, as we had previously, that both hospitalized patient groups (underweight anorexics and normal-weight bulimics) had significantly more pathological scores on the MMPI compared to either normal controls or long-term weight-restored anorexics. Specifically, the underweight anorexics had higher scores on the Hypochondriasis, Depression, Hysteria, Psychasthenia, and Social Introversion scales compared to the normal-weight bulimics. On the cognitive tests, underweight anorexics and normal-weight bulimics showed deficits on a number of tasks assessing aspects of attention (Continuous Performance Test, Letter Cancellation Test). In addition, some deficits were seen on tests of verbal memory; these memory deficits disappeared, however, when the contribution of attentional problems

in these tasks was controlled statistically. On a number of tests, however, significant deficits remained: The underweight anorexics performed significantly worse than all other groups on the Comprehension subtest of the WAIS-R and worse than the normal controls on the Similarities subtest; on the Arithmetic subtest of the WAIS-R, the normal-weight bulimics were impaired; and finally, on non-verbal learning, as assessed by the Recurring Figures Test, the underweight and long-term weight-restored anorexics were impaired, particularly those who had lost weight by restricting caloric intake (restrictor type) as opposed to those who had engaged in binge eating and vomiting or using laxatives (bulimic type).

Upon retest following weight restoration, the elevated scores on the MMPI exhibited by the underweight anorexics were significantly reduced; however, differences from the normal control group remained. On most cognitive tests, improvement was seen, some of which could have been due to the effects of practice.

2. Affective Disorder Patients

Recent developments in the study of affective disorders and their treatments have suggested a rationale for examining the similarities and differences between patients with bipolar affective illness and patients with complex partial seizures. Post, Ballenger, and their colleagues have proposed a kindling model of affective illness and temporal lobe epilepsy. Briefly, it is postulated that repeated seizures (in the case of temporal lobe epilepsy) or repeated biochemical and/or psychological stresses (in the case of affective illness) produce cumulative bioelectrical changes, which in turn result in abnormal limbic neuronal sensitization and major psychiatric disturbances. Both disorders are thought to involve temporal lobe and limbic system abnormalities, and both disorders have been shown to respond to treatment with anticonvulsant agents, including carbamazepine.

We are in the process of examining the neuropsychological profiles of patients with complex partial seizures, patients with bipolar affective illness, and normal controls. Preliminary analyses of the neuropsychological test data of 11 complex partial seizure patients, 13 patients with bipolar affective illness (tested on placebo during their first test session), and 15 normal controls show that the complex partial seizure patients perform significantly more poorly than either patients with bipolar affective illness or normal controls on a number of neuropsychological measures. These measures include the Trail Making Test, the Complex Figure Test, the Purdue Pegboard Test, the Buschke Selective Reminding Procedure, and the Stroop Test (Word, Color, and Color-Word measures). In the results for the Trail Making Test, in addition to the significant group differences, there was also a significant interaction between task and group. There were no significant group differences on the Continuous Performance Test, the Halstead Category Test, or the Stroop Interference measure. However, because there were significant group differences on Full Scale and Verbal IQ (with the complex partial seizure patients scoring lower than the other two groups) and on Performance IQ (with the complex partial seizure patients lower than the controls), we

performed analyses of covariance using Full Scale IQ as the covariate to see what group differences in cognitive measures remained when the (probably bona fide) IQ differences were controlled. When these analyses of covariance were performed, significant main effects of group remained only for the Trail Making Test. These findings are of interest and merit further investigation. We plan to increase the sizes of our samples, and we are particularly interested in testing more high functioning complex partial seizure patients in order to see how these patients might differ from our rather high functioning bipolar affective disorder group.

3. Children with Attentional Disorders

This project is a collaborative effort with the Prevention Intervention Research Center (PIRC), a program of the Department of Mental Hygiene of the Johns Hopkins School of Hygiene and Public Health and the Baltimore City Public Schools. The PIRC study involves children in the first and second grades of the Baltimore Public School System who are at risk for the development of substance abuse, delinquency, and/or psychopathology, as well as a large group of normal controls. Two cohorts (approximately 2400 children) of first-grade children attending 19 elementary schools in Eastern Baltimore are being assessed periodically through teacher ratings, peer nominations, independent behavior time sampling, and structured self-reports coupled with information on school progress.

Our interest in this population has been the neuropsychological assessment of attention. The correlation between attention deficit disorder and the later development of substance abuse, delinquency, and various forms of psychopathology has been documented in the literature. Furthermore, the taxonomy of attention and the relationships between impairments of the various components of attention and classroom learning, behavior, and the later development of psychopathology are of considerable interest in their own right. During the past year, we have developed, tested, and implemented a battery of neuropsychological tests of attention. This battery has been designed not only to assess attention but also to separate more global "attentiveness" into different components of attention. Previous work from the LPP, examining attentional components in adults, delineated a focus/execute aspect, tapping perceptual-motor speed, a sustain component involved in vigilant behavior, an encode factor, capturing numerical-mnemonic qualities of attention, and the flexibility aspect of attention or the ability to shift focus. In developing the battery for children, we borrowed some tasks used in the adult work, adapted others for use with children, and added some new instruments. In its final form, the battery consisted of three versions of the X Task of the Continuous Performance Test (standard, using auditory distraction, using degraded visual stimuli), Digit Cancellation (with and without auditory distraction), the Wisconsin Card Sorting Test, three subtests of the WISC-R (Coding, Arithmetic, Digit Span), and the Peabody Picture Vocabulary Test.

We piloted the one-hour battery on 50 second graders in a school not participating in the PIRC program. Preliminary analysis of the data indicates

the presence of a sustain factor, reflected by Continuous Performance Test accuracy and a shift factor comprised of performance on the Wisconsin Card Sorting Test. In adults, the focus aspect of attention was intertwined with an execute, or speed, component. In children, these components were found to be distinct, reflected by Digit Cancellation performance and speed of Continuous Performance Test responses, respectively. Of interest was a major impulsivity factor that appeared in the child data, consisting of errors of commission on the Continuous Performance Test and Digit Cancellation Test. In the spring of 1987, this battery was administered to a representative sample of 435 second graders participating in the PIRC project. The data are currently being scored, coded, and prepared for analysis. These data will provide the first information on the incidence of neuropsychologically-evaluated attentional deficits in a population-based sample. In addition, plans call for the data to be correlated with other information collected on these children to assess the relationship of such deficits to teacher ratings of attention, learning, and behavior in the classroom and to various maladaptive behaviors. Also, we plan to follow these children to examine the usefulness of the battery in the prediction of future disordered behavior.

4. Inherited Metabolic Disorder Patients

A project is underway to study the neuropsychological status of children with inherited metabolic disorders. Two disorders are currently under study: Gaucher's disease, Type I and III; and cystinosis, infantile type.

A subset of the LPP neuropsychological test battery has been selected and is being administered serially to assess the presence, if any, of intellectual deterioration over time. Also to be studied are the neuropsychological profiles of the various groups, as well as the presence of signs consistent with focal brain lesions. Control subjects for the Gaucher's disease group will be drawn from the LPP-Johns Hopkins PIRC collaborative project, as our largest group of subjects in this study is in the age range of 5 to 9 years.

A number of different clinical subgroups can be distinguished in Gaucher's disease. We are studying those forms in which a slow, progressive deterioration in the central nervous system (CNS) has been identified, namely, Types I and III. Within Type I, a further distinction is made between Jewish and non-Jewish patients, since thus far there is no evidence of CNS deterioration in Jewish patients. So far, 17 patients with Gaucher's disease have been studied once; follow-up data are not yet available on any of the patients.

We have studied 8 cases of cystinosis of the infantile type who have had kidney transplants following chronic renal failure. There is some evidence that slow, progressive neurologic deterioration may occur following long-term survival with this disease. This is thought to be due to CNS pathology. Preliminary analysis of our data does not reveal evidence of deterioration and thus suggests that the incidence of intellectual loss is low.

5. Closed Head Injury Patients

The LPP has begun a study of patients with closed head injuries who are referred by the Maryland Head Injury Foundation. This study is designed primarily to provide heuristic models for psychiatric illnesses based upon information from subjects with cerebral lesions. In addition, the data will allow validation of the cerebral localizing value of tests in our neuropsychological battery. Portions of the neuropsychological battery have been shown in previous studies to be sensitive to frontal-lobe damage, temporal-lobe damage, brainstem damage, and left- and right-hemisphere damage, respectively. However, cross-validation studies are needed; and, to this end, we are seeking to test six classes of patients at this time:

- a. bilateral frontal-lobe damage
- b. bilateral temporal-lobe damage
- c. bilateral frontotemporal-lobe damage
- d. brainstem damage
- e. diffuse right-hemisphere damage
- f. diffuse left-hemisphere damage

It had been our original plan to test patients with damage confined to one lobe of the brain (e.g., left or right frontal, left or right parietal, etc.); however, it has become clear that the nature of closed head injuries is such that damage confined to one lobe of the brain is very rare. At the same time, patients with more widespread damage are of considerable interest in themselves and of considerable relevance to the study of psychopathology, since the presumed cerebral dysfunction underlying a number of forms of psychopathology is likely to involve more than one lobe of the brain. In addition, although much is already known about the performance of these kinds of patients on neuropsychological tests, we have not as yet had the opportunity for a cross-validation study using our particular battery of neuropsychological measures. This study will provide such an opportunity.

Thus far we have completed neuropsychological testing of 7 closed head-injury patients: 3 with bilateral frontotemporal damage, 1 with bilateral frontal-lobe damage, 1 with brainstem damage, and 2 with diffuse right hemisphere damage. As a preliminary look at these data, we have compared the neuropsychological test performance of the 3 patients with bilateral frontotemporal damage and the patient with bilateral frontal-lobe damage to a group of hospitalized schizophrenic patients, since it has been hypothesized by some that the locus of cerebral dysfunction in schizophrenia is in bilateral frontotemporal areas. Preliminary analyses reveal that, as expected, the schizophrenics had more elevated scores than the head-injury patients on the MMPI, with significantly higher scores on the Frequency, Hypochondriasis, Psychasthenia, Schizophrenia, and Social Introversion scales. However, the schizophrenics also performed significantly worse than the head-injury patients on a number of cognitive measures, including the memory passages from the Wechsler Memory Scale, part B of the Trail Making Test, immediate and delayed recall of the Complex Figure Test, the Color and Word conditions of the Stroop Test, WAIS-R Performance IQ, and the WAIS-R

subtests Arithmetic, Picture Completion, and Object Assembly. Finally, the schizophrenics performed significantly worse than the bifrontal or bilateral frontotemporal patients on three Wisconsin Card Sorting Test measures (total errors, perseverative errors, and conceptual level responses). We will be increasing the sample sizes of these groups in order to determine whether these very preliminary but interesting and somewhat unexpected findings hold up.

Proposed Course

We are planning to increase our sample sizes for several of the ongoing studies, in order to provide greater power to our analyses. These studies include those of eating disorders; Gaucher's disease; normal children and children at risk for the later development of substance abuse, delinquency, and/or psychopathology in the LPP-Johns Hopkins collaborative study; schizophrenia; and patients with closed head injuries. In addition, we plan to increase the number of normal controls assessed using all forms of the neuropsychological batteries to facilitate comparison with the various patient groups. A number of normal controls will also receive follow-up neuropsychological testing in order to allow for the analysis of data in studies that involve repeated neuropsychological testing. During the coming year, we plan to begin a more intensive study of the correlations between the neuropsychological test data and electrophysiological data that are gathered concurrently on many of these patient populations as well as the relationship to biochemical measures.

Publications

Joffe, R.T., Rubinow, D.R., Squillace, K., Lane, C.H., Duncan, C.C., and Fauci, A.S.: Neuropsychiatric aspects of AIDS. Psychopharm. Bull. 22: 684-688, 1986.

Mirsky, A.F., and Duncan, C.C.: An introduction to modern techniques of clinical neuropsychology. In Fava, G.A., and Wise, T.N. (Eds.): Research Paradigms in Psychosomatic Medicine. Basel, Karger, 1987, pp. 167-184.

Jones, B.P.: Advise and dissent [Review of Clinical Application of Neuropsychological Test Batteries]. Contemp. Psychol., in press.

Jones, B.P.: Updating for clinical neuropsychologists [Review of Neuropsychological Assessment of Neuropsychiatric Disorders]. Contemp. Psychol., in press.

Jones, B.P., Henderson, M., and Welch, C.A.: Executive functions in unipolar depression before and after electroconvulsive therapy. Int. J. Neurosci., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00509-05 LPP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Attention Disorders As Assessed by Event-Related Brain Potentials

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Connie C. Duncan, Ph.D., Chief, Unit on Psychophysiology, LPP, NIMH

COOPERATING UNITS (if any) Clinical Psychobiology Branch, Neuropsychiatry Branch, Laboratory of Clinical Science, Child Psychiatry Branch, NIMH; Chestnut Lodge Hospital; Developmental Neurology Branch, Medical Neurology Branch, NINCDS; Maryland Head Injury Foundation.

LAB/BRANCH

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TOTAL MAN-YEARS:

4.0

PROFESSIONAL:

0.8

OTHER:

3.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project is to investigate the roles of event-related brain potentials, attention, and information processing and their interrelationships in the etiology, pathology, and prognosis of psychiatric and neurological disorders. Major emphasis is on the diagnostic specificity of disorders of attention and cognition and the identification of the specific aspects or stages of information processing underlying observed decrements in performance. Concurrently recorded event-related brain potentials and performance on cognitive tasks are used to define mechanisms of cognitive failure in subjects with diagnoses of seasonal affective disorder, schizophrenia, eating disorders, learning disorders, seizures, and closed head injury. Event-related brain potentials are also used to investigate the role of altered neurochemical mechanisms by comparing drug-induced electrophysiological and behavioral effects with those seen in the various disorders. Psychological correlates are investigated by relating the data to extensive neuropsychological, psychiatric, and personality measures as well as to performance on behavioral tasks.

Project DescriptionA. Other Personnel

Allan F. Mirsky, Ph.D.	Chief	LPP, NIMH
Barbara P. Jones, Ph.D.	Special Expert	LPP, NIMH
Edward Turner, M.S.W.	Research Social Worker	LPP, NIMH
Norman E. Rosenthal, M.D.	Chief, Unit on Out- Patient Studies	CPB, NIMH
Robert G. Skwerer, M.D.	Medical Staff Fellow	CPB, NIMH
Darrell G. Kirch, M.D.	Associate Clinical Director	NPB, NIMH
Ralph W. Fawcett, M.D.	Medical Staff Fellow	NPB, NIMH
C. Wesley Dingman, M.D.	Assistant Clinical Director	Chestnut Lodge Hospital
David C. Jimerson M.D.	Chief	SBP, LCS, NIMH
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B. Objectives

The major objective of this project is to yield data that will illuminate the neurophysiological bases of the cognitive and attentional deficits in the clinical disorders of seasonal affective disorder, schizophrenia, eating disorders, dyslexia, epilepsy, closed head injury, and other forms of brain pathology. Defining the specific ways in which information processing can fail may provide new diagnostic strategies for more effective evaluation and treatment of patients with attentional and cognitive impairments. A related objective of this project is to differentiate state versus trait attributes of these disorders to increase understanding of their etiologies. Concurrently obtained event-related brain potentials (ERPs) and measures of performance during active cognitive processing are used to define the mechanisms of attention failure in these syndromes. Defining and understanding the different determinants and forms of attentional and cognitive failure is diagnostically important as well as useful in characterizing the nature of the psychobiology of attention disorders. Finding differences in overall response levels between normal subjects and patients is a necessary first step; however, the goal is to use the knowledge to lead to new approaches to classification and treatment, to increased understanding of etiology, and to effective preventive interventions.

C. Methods Employed1. Electrophysiological Assessment

The general methods of these studies include recording the EEG, using the International 10/20 system, from frontal (FPz, Fz, F3, F4), central (Cz, C3,

C4), parietal (Pz, P3, P4), and occipital (Oz) scalp sites referred to linked ears, while stimuli are presented to the subject. Stimulus presentation and data collection are controlled by a PDP-11/34 or PDP-11/73 computer. Eye movements are monitored during the recordings, and trials contaminated by artifacts are discarded. The EEG is averaged to yield ERPs. Since these scalp-recorded electrical waves are associated in time with either an event in the environment; such as the presentation of a stimulus, or with an internal cognitive event, they are called event-related brain potentials.

Brainstem auditory evoked responses (BAERs) are used to measure, directly and noninvasively, the progress of a sensory signal through brainstem to the cortex, and thereby obtain a measure of the integrity of brainstem functioning. BAERs are obtained by presenting click stimuli to the ears.

Evaluation of endogenous components of the ERP, associated with higher-level processes such as selective attention, learning, memory, and decision-making, yields information on the attentional and cognitive functioning of the subject. The ERPs are elicited by trains of auditory or visual stimuli presented in the context of an attentional or cognitive task. The selection of tasks, which use reaction time techniques as well as recall and recognition of stimulus material, allows for the measurement of a pattern of cognitive behaviors and associated ERPs, where different components reflect different aspects of information processing. Using ERPs, it is possible to get an indication of the subject's processing of all environmental stimuli, both relevant and irrelevant, and thus to assess, for example, the differential processing that is the hallmark of selective attention.

A major focus of our investigations is the "P300" component of the ERP. This scalp-derived electrical potential appears 300 msec or longer after an event that engages the interest or attention of a subject and is a positive voltage as recorded on the scalp; hence the name P300. The amplitude of the P300 component depends on the amount of processing capacity invoked by a stimulus and reflects stimulus-evaluation and decision-making activity. It is also a sensitive indicator of orienting reactions to novel, surprising, or incongruous stimuli and a predictor of the memorability of events. Moreover, P300 allows a direct evaluation of the subjective probabilities that a subject assigns to event outcomes and may reflect the extent to which a stimulus is encoded. The latency of P300 indexes the time required to classify and evaluate a stimulus independent of response-production factors. ERPs can thus help to clarify the timing and order of neural events in information processing activities and to identify the aspects or stages of information processing responsible for observed decrements on cognitive tasks in a variety of clinical populations.

2. Neuropsychological Assessment

Normal volunteers are screened by a psychologist who uses the lifetime version of the Schedule for Affective Disorders and Schizophrenia (SADS-L) to exclude those with past or current psychopathology or with first-degree relatives with such a history. Many patients and normal volunteers are

evaluated on an extensive neuropsychological battery of cognitive and sensory functioning. When appropriate, tests of formal thought disorder are administered. We plan to correlate the neuropsychological and electrophysiological data to aid in the classification of disorders characterized by attentional deficit and cognitive failure.

3. Biological Assessment

In collaboration with other laboratories, patients are assessed for treatment responsiveness. In some studies, blood, urine, and/or cerebrospinal fluid measurements reflecting neurochemical activity are correlated with electrophysiological, neuropsychological, and behavioral data. We also plan to use X-ray transmission tomography (CT scan) to measure ventricular size. These data will be correlated with electrophysiological data to yield information on the relation between ERPs and cerebral structures.

D. Major Findings

We are using a variety of attentional paradigms, which tap visual and auditory information processing systems, to investigate patients with seasonal affective disorder, schizophrenia, eating disorders (anorexia nervosa and bulimia), adult dyslexia, absence epilepsy, and closed head injury. These tests provide a differential assessment of specific types of attention, including the ability to initiate, select, inhibit, shift, and sustain attention. The protocol also includes evaluation of automatic and controlled cognitive processes. The rationale for the approach of using the same paradigms, which tap specific cognitive processes, on different patient groups is to allow inferences about which processes are uniquely impaired in one group in comparison with other groups. To determine whether ERPs can serve as sensitive yet specific markers of disorder, patients with diverse symptomatologies and diagnoses are compared. The ERP measures are correlated with concurrently recorded behavioral responses, including reaction time, and in some studies, with performance on neuropsychological tests.

A number of ERP studies have been completed during the past year, and the results are now either in press or are being analyzed in preparation for publication. The studies include investigations of seasonal affective disorder, schizophrenia, eating disorders, the alpha-2 adrenergic agonist clonidine, dyslexia, and absence epilepsy. The results of each of these studies are summarized briefly below.

1. Seasonal Affective Disorder

Seasonal affective disorder (SAD) is a syndrome characterized by recurrent depressions during the fall and winter months, when daylight is at a minimum. The symptoms of SAD, including decreased activity, sadness, irritability, anxiety, and appetite changes, can be treated with bright artificial light. Moreover, the depression remits spontaneously when daylight increases in the spring. We used ERPs to investigate whether improvement in clinical state following phototherapy is associated with an increase in the attentional

resources allocated to the processing of stimuli in the visual modality. We wished to learn whether P300 changes would reflect clinical improvement. Such a result might increase our understanding of the pathophysiology of this disorder.

Subjects were 21 patients (15 female) who met the criteria for SAD. The patients were tested twice, once following nine or more days of phototherapy ("On Lights") and once either preceding phototherapy or nine or more days following its cessation ("Off Lights"). Order of the two tests was counterbalanced across subjects. Light therapy comprised two 2 1/2-hour sessions of 2500-lux lights per day, one in the morning and another in the evening. ERP testing followed immediately after the morning phototherapy. A subset ($n = 11$) of the control subjects was also tested twice under the same experimental conditions. Clinical response was assessed with the Hamilton Rating Scale for Depression.

Between-subject correlations on the patient data from the first year of the study were computed to determine the relation between the change in P300 amplitude and the change in clinical ratings following phototherapy. SAD patients who exhibited the most clinical improvement showed the greatest increase in P300 amplitude in the visual modality ($r = -.85$, $p < .005$). In contrast, clinical response was uncorrelated with changes in P300 to auditory stimuli ($r = .24$). Moreover, neither the speed nor accuracy of performance correlated significantly with changes in the clinical ratings. No changes in P300 were apparent in either modality in the two tests of the controls. It thus appears that light treatment increases the amplitude of the P300 component in direct proportion to its antidepressant effect.

The time course of this effect was evaluated in 6 patients and 2 controls who were tested several times during light therapy as well as at baseline. The enhancement of P300 was found to occur as early as 2 days after the initiation of light treatment in SAD patients. This finding is of considerable interest in that it provides, for the first time, an objective, sensitive index of the antidepressant effects of light. The results suggest that the response to phototherapy in SAD patients is highly correlated with an increase in the attentional resources that are mobilized to process visually-guided information. It may be that the favorable clinical response to phototherapy is in part mediated by enhanced cognitive capacities of SAD patients.

2. Schizophrenia

During the past 15 years, a number of studies have shown that the amplitude of the P300 component of the ERP is reduced in schizophrenic patients. The amplitude of P300 has been shown to be a sensitive index of attention deployment, so that its reduction in schizophrenic patients is consistent with the behavioral findings. However, since the P300 is derived from cerebral electrical activity, it offers the potential to study dynamic brain function. Thus, the P300 component is an attractive tool to investigate putative neurobiological mechanisms underlying the attention deficit in

schizophrenia. We extended and broadened the finding of attenuated P300 in schizophrenic patients by evaluating the relative effects of stimulus modality and probability.

A total of 58 patients (including 24 unmedicated) who met DSM-III Criteria for schizophrenic disorder were matched on age, sex, and education to a group of 24 normal controls. Preliminary analysis of the data showed that, as in previous studies, the P300 was smaller in the schizophrenic patients than the normal controls. However, this difference was significant only for low probability stimuli in the auditory modality and was not found to be significant in the visual modality, suggesting that schizophrenic patients have a greater deficit in auditory than in visual processing. This finding may provide a clue to the underlying pathophysiology and is reminiscent of the relative prevalence of auditory as compared with visual hallucinations in schizophrenic symptomatology.

We sought to determine whether this P300 reduction observed in schizophrenic patients is a reflection of a core deficit, independent of clinical state, or whether it is a reflection of clinical symptomatology. That is, is the reduced P300 a trait as contrasted with a state marker of the disorder? To address this question, 11 schizophrenic patients were tested twice, once when they had been free of medication for at least 4 weeks and once when they had been stabilized on neuroleptics for at least 6 1/2 weeks. To control for the effects of repeated testing, 7 matched normal controls were also tested twice, at approximately the same intervals as the patients.

Clinical state was assessed with the Brief Psychiatric Rating Scale. The relation between the change in P300 amplitude and the clinical response to medication was assessed in the first several patients. Patients who exhibited the most clinical improvement showed the greatest increase in P300 to visual stimuli ($\bar{r} = -.88$, $p < .01$). In contrast, clinical response was uncorrelated with changes in P300 to auditory stimuli ($\bar{r} = .13$). No change in P300 was apparent in either modality between the first and the second evaluation of the controls.

The increase in visual but not auditory P300 amplitude is consistent with the hypothesis that successful neuroleptic treatment enhances a patient's capacity to process visual but not auditory information. Because auditory P300 amplitude was not correlated with clinical state, it remains a candidate for a vulnerability trait marker of schizophrenia. Our data, in fact, suggest that auditory P300 appears to be significantly more sensitive to differences between schizophrenic and normal persons than is visual P300. Moreover, it is clear that mere treatment with neuroleptic medication alone, without symptomatic change, is insufficient to alter visual P300 amplitude. It is conceivable that the core deficit in schizophrenia is more closely related to relatively invariant impaired auditory information processing and that fluctuations in clinical symptomatic state are reflected in visual processing.

3. Eating Disorders

Persons with eating disorders appear to be characterized by altered cognitive processing. In particular, there have been reports that patients with anorexia nervosa may be impaired in automatic but not in controlled processing. We used ERPs to assess these aspects of information processing.

All patients were women who met DSM-III criteria for anorexia nervosa ($n = 24$) or normal weight bulimia ($n = 34$). A group of 27 matched normal controls was also studied. Auditory and visual versions of four reaction time tasks, which varied along the automatic-controlled dimension, were employed to elicit ERPs.

The results indicate separation on the ERP measures between anorexic and control and between anorexic and bulimic subjects. Specifically, anorexic patients showed disturbances in automatic processing, as indexed by a component reflecting an automatic cerebral mismatch process. Altered controlled processing, as measured by P300 amplitude, was also seen in the anorexics; the difference increased with increasing task demands. Bulimics were not distinguishable from controls on the measures. Preliminary findings after long-term weight restoration in 15 anorexic patients indicate reversal in all of the ERP abnormalities except one: The Slow Wave component following P300 was significantly enhanced in these patients, indicating a relatively permanent disturbance in controlled information processing.

4. Clonidine

A number of investigations have found that malnourished, underweight patients with anorexia nervosa have decreased concentrations of norepinephrine or MHPG in urine, plasma, or CSF. More recently, several studies have suggested that underweight anorexic patients have increased alpha-2 adrenoceptor activity. Such findings suggest that the functional activity of noradrenergic systems is reduced in these patients. A major CNS noradrenergic pathway originates in the locus ceruleus, which, by virtue of its widespread cortical and subcortical connections, appears to play a role in the maintenance of such functions as attention, sleep, and wakefulness. We have demonstrated that there is an attentional deficit in women with anorexia nervosa that is reflected in changes in the ERP. This study was designed to explore the possible role of locus ceruleus pathophysiology in the attentional disturbance in anorexia nervosa. Clonidine, a relatively specific alpha-2 adrenoceptor agonist, is reported to decrease locus ceruleus activity. This drug was given to healthy, normal women to determine whether it would produce ERP changes similar to those observed in anorexic patients. Such changes would support the hypothesis of a disturbance in locus ceruleus function in this disorder.

Eight healthy young women were administered, on separate days, three doses of clonidine (0.5, 1.0, and 2.0 micrograms/kilogram) and two saline placebos infused intravenously in a counterbalanced order under double-blind conditions. To assess effects on attention, ERPs were recorded from a variety

of standard scalp placements during auditory discrimination tasks.

Results indicate that clonidine produced changes in the ERP that resemble some of the alterations observed in anorexic patients, namely, decreases in the amplitude of the P300 component. The data thus support, in part, the hypothesis of altered locus ceruleus function in anorexia nervosa. However, the lack of effect of clonidine on the early negative component suggests that neurochemical systems other than the noradrenergic locus ceruleus system may underlie the reduction in this component in anorexic patients.

5. Dyslexia

Sixteen dyslexic adult men, including 7 with a probable history of Attention Deficit Disorder (ADD) and 8 with no history of Attention Deficit Disorder (NONADD), and 15 matched normal controls were studied with a battery of paradigms designed to elicit ERPs. The goal of the study was to discover whether dyslexic adults differ from controls in visual and/or auditory information processing.

Smaller P300s were observed over the left hemisphere for both patient groups in comparison to controls. Over the right hemisphere, P300 amplitude of the NONADD group was equal to that of the controls, while the ADD subjects' P300s remained relatively attenuated. No group differences in modality were found. P300 latency in the parietal areas was longer for both the ADD and NONADD subgroups relative to the controls, but these differences were not specific to one modality. The amplitude of the auditory Slow Wave was smaller over both hemispheres for ADD than for NONADD or control subjects, while the NONADD subjects exhibited larger visual and auditory Slow Wave components over the right hemisphere. This difference between groups diminished with decreasing attentional load.

The reduced amplitudes of the P300 and Slow Wave components for the ADD group suggests a generalized deficit. Although these results must be considered as preliminary, they suggest that different etiologies may underlie dyslexias with and without concomitant attention deficit. Both dyslexic groups showed evidence of compromised left hemisphere functioning; however, the possibility exists, as well, of greater right hemisphere (and right parietal) involvement in dyslexia with ADD. Such considerations suggest two different types of pathophysiological processes underlying the two disorders and may ultimately lead to different strategies of remediation in the two groups of patients.

6. Absence Epilepsy

The ERP technique was used to study information processing in the interictal period in a group of 8 patients with absence seizures and a matched group of 8 normal controls. Event-related brain potentials were recorded during performance of auditory and visual versions of the Continuous Performance Test (CPT) of sustained attention.

Reaction time differences on the CPT between the absence patients and the normal controls confirm previous findings: such patients are strikingly impaired in their ability to perform tasks of sustained attention. This study extended the previous findings by demonstrating convincingly that the attention deficit is observed in the auditory as well as the visual modality. The behavioral differences seen on the CPT were paralleled in virtually isomorphic fashion in the P300 amplitude data. Whereas the between-group differences in visual P300s were somewhat smaller, the between-group P300 differences found in both versions of the auditory CPT were substantial. Moreover, the between-group processing differences apparent in the ERPs to target stimuli were also seen in the ERPs to at least one category of nontarget trial--warned, no-go trials. These waveforms suggest, in fact, that the control subjects' processing of this category of nontarget stimulus was at least as complex as their processing of target stimuli: the P300 and Slow Wave components elicited under these conditions were larger than in the other conditions in this experiment. Such waveform complexity was either not present in the absence patients or was present in a much attenuated form.

N100 amplitude and latency were also evaluated as additional indices of information processing. Whereas N100 amplitude did not vary between groups, N100 latency was longer for the absence patients than the normal controls.

Although the possibility of a generalized deficit in the patients seems unlikely, this possibility was tested by computing average ERP waveforms for the three highest functioning patients only. These average ERPs were essentially the same as those based on the remaining patients. Moreover, analyses of the P300 data of this subgroup of patients and a matched subgroup of three normal controls yielded the same results as the analyses based on the full samples.

This suggests that the failure to respond efficiently on visual and auditory forms of the CPT is due, at least in part, to the failure of absence patients to mobilize and sustain attentional capacity. Whereas later N100s are indicative of delayed perceptual encoding, the lack of difference in P300 latency indicates that the higher-level processes of identifying and categorizing the stimulus proceed at an approximately normal rate. The reaction time data indicate a significantly slower response to auditory targets in the patients. The lack of a significant difference between groups in P300 latency suggests that the slower response is not due to an increase in stimulus processing time but to prolonged response processing in the patients. Moreover, the lower percentage of correct responses in the patients indicates that this prolonged response processing is not due to the use of a more cautious response strategy. Rather, it appears that the execution of the response itself is delayed. Thus, by using P300 in conjunction with reaction time, we were able to show that the delay in responding on the auditory CPT-AX task exhibited by patients with absence epilepsy occurs subsequent to stimulus evaluation.

Significance to Biomedical Research and the Program of the Institute

Since attentional deficit and cognitive dysfunction are characteristic of many psychopathological and neuropathological disorders, it is important to develop a precise empirical and theoretical account of these symptoms. The scalp-recorded ERP is the only noninvasive technique available for studying the dynamic neural activity associated with cognitive processing in human subjects. The ERP provides information on mental events involved in selective attention, stimulus evaluation and decision making, memory, learning, and response preparation. The temporal resolution of ERPs can support inferences about brain activity on time scales not possible in studies using tissue assays or radioactivity. Because of the noninvasive character of ERPs, patient state can be monitored often enough to assess the effects of specific clinical or experimental variables. The appropriateness of evaluating ERPs in studies of attention is apparent, as they may provide a dissection of the various components involved and thereby permit more precise identification of the types of information processing deficits responsible for poor performance on attention tasks in a variety of patient groups. It is hoped that the developing battery of ERP and neuropsychological tests applied to patients characterized by attentional deficit and cognitive dysfunction will ultimately provide a neurobiological profile of each disorder and lead to more refined subcategorizations, as well as to more efficacious treatments.

Proposed Course

We are currently completing data collection on our studies of patients with eating disorders. Data analysis is in progress on our studies of seasonal affective disorder, schizophrenia, absence epilepsy, dyslexia, and clonidine. We plan to continue our investigations of patients with seasonal affective disorder, schizophrenia, and closed head injury. We plan to extend our studies of affective disorder patients in depth as well as scope. In the seasonal population, we are interested in whether an even more rapid change in P300 may be measurable. If so, this would be of predictive value in evaluating which patients are most likely to benefit from light therapy. It would also be of theoretical value in helping to evaluate the time course of the neurobiological processes underlying the effect of phototherapy. In addition, we plan to study P300 in other types of affective disorder patients to assess the specificity of our findings. Our work is aimed at illuminating the neurophysiological bases of the cognitive and attentional deficits in affective disorder, schizophrenia, and other psychiatric disorders. Of interest are the relation of ERP variables to diagnosis, diagnostic symptomatology, severity of disorder, degree of formal thought disorder, performance on tests of attention, memory, and intellectual functioning, degree of improvement during treatment, and improvement on specific treatments. We plan to expand our investigation of the interrelation among ERP components and neuropsychological and neurochemical variables. To increase our understanding of the etiology of schizophrenia and affective disorder, we are planning additional studies to differentiate state versus trait attributes of the disorders. The strategy we intend to use is to compare normal controls with patients when they are actively symptomatic and

when they are in remission. We plan to begin testing first-degree relatives of psychiatric patients to determine whether the ERP is a marker of specific disorder. Selected studies with head-injured cases are planned to test hypotheses derived from various clinical groups concerning the involvement of brain structures in the pathophysiology of psychiatric disorders. Electro-physiological predictors of clinical response to psychopharmacological and other forms of treatment will be sought, as patient availability allows.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

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PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Etiological Factors in Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of the occurrence of mental illness in families have been useful in identifying familial forms of the illnesses and in the development of hypotheses regarding the form and strength of genetic and environmental factors in etiology. Where these major variables are separated by the process of adoption, specific etiologic hypotheses can be tested separately and in combination. A total national sample of 14,500 adult adoptees in Denmark provides the basis of the main components of this research. The research this year has focused on 46 classical schizophrenic adoptees identified in the national sample with a comparable number of control adoptees with no history of mental illness. The remarkable population registers in Denmark permit the identification of the close biological and adoptive relatives of these adoptees. By search of mental hospital registers and ultimately by personal interviews, information on the psychiatric history and status of the relatives has been obtained. During the past year these interviews and other records have been used to obtain consensus diagnoses by two raters without knowledge of the relationship among relatives and adoptees. The first phase of the analysis has now been completed with results which confirm the findings on the first sample (restricted to Greater Copenhagen) that classical chronic schizophrenia occurs almost exclusively among the biological relatives of chronic schizophrenic adoptees and not in their adoptive relatives. A marginal syndrome which has been variously designated as latent or borderline schizophrenia or schizotypal personality was also found to be significantly more prevalent in the biological relatives of chronic schizophrenic adoptees, but was also more prevalent in the biological relatives of adoptees with other major mental disorders or marginal schizophrenia.

The study of mental disorder in the relatives of adoptees with affective disorder completed in the previous year has now been published.

Project Description

Objectives

The objective of this phase of the study of schizophrenic adoptees and their families has been to extend the survey, initially confined to the city and county of Copenhagen, to all of Denmark, evaluating the strength of genetic and family-related environmental influences, and to define more explicitly the traits which comprise the syndrome of latent schizophrenia.

Adoption Study of Schizophrenia

Comprehensive interviews with the biological and adoptive relations of 34 schizophrenic adoptees and 34 non-schizophrenic control adoptees, rated blindly, had found a significantly higher prevalence of schizophrenia and schizophrenia-related disorder in the biological relatives of schizophrenic adoptees than in controls. Moreover, eight of the characteristics found in the relatives diagnosed by us as latent or uncertain schizophrenia permitted us to characterize them with the DSM-III diagnosis of "schizotypal personality disorder."

In the 9,000 adoptees outside of Copenhagen, 37 were identified as having developed schizophrenia, of which 29 qualified as 'chronic schizophrenia' as described in DSM-II or 'schizophrenia' in DSM III. In the course of the past three years, interviews were completed on approximately 90 percent of the relatives of these and their control adoptees who are alive and residing in Denmark; these interviews were rated blindly by two experienced judges using global evaluations based on the descriptions of Kraepelin and Bleuler.

During the past year those evaluations were completed and complemented by a review of abstracts of hospital records and reports of incomplete interviews. As was the case in the Copenhagen sample, over 90% of the relatives alive and residing in Denmark, Sweden or Norway participated in a 36 page interview covering a social, psychological and medical history and a complete mental status examination. Since the research design called for control adoptees with no history of serious mental illness, the initial group, selected as having no record of hospitalization for mental illness, was also interviewed and rated blindly along with the relatives. The 26 control adoptees thus selected gave complete interviews and no mental disorder more serious than anxiety neurosis or mild depression was found.

The study in Copenhagen found a significant concentration of schizophrenia and schizophrenia-like disorders (chronic, acute, latent and probable schizophrenia, and schizoid personality - the 'schizophrenia spectrum of disorders') in the biological relatives of the chronic schizophrenic adoptees. The same finding occurred in the present or Provincial sample representing the rest of Denmark outside of Copenhagen, (34 among 173 identified biological relatives of chronic schizophrenic adoptees versus 11 among 162 such relatives of the control adoptees, $p = 0.0004$). Further

breakdown of the schizophrenia spectrum in the present sample gave results which confirmed those from the Copenhagen study: 'acute schizophrenia' (a diagnosis similar to DSM-III schizophreniform disorder) and 'schizoid personality' did not occur significantly more often in the biological index relatives than in those of the controls; if these two categories are excluded from the spectrum, its preponderance in the biological relatives of the chronic schizophrenic (index) adoptees is considerably enhanced as it was in the Copenhagen sample (24/173 vs 3/162 $p = 0.00003$). Chronic schizophrenia appeared only in the biological index relatives (5/173 vs. 0/162, $p = 0.036$). Probable chronic schizophrenia as well was absent from the relatives of the controls and if the results for definite and probable schizophrenia are added the results are even more striking (9/173 vs 3/162 $p = 0.008$). Latent schizophrenia was found three times more frequently in the relatives of latent schizophrenic adoptees than in those of chronic schizophrenic adoptees.

These vaguer syndromes are not limited to the index relatives, but, in contrast to chronic schizophrenia, are found in comparable prevalence in the biological relatives of adoptees who could not be interviewed or were rejected as controls by virtue of a history of serious mental disorder (major affective, hysterical psychosis, or a schizophrenia spectrum disorder). This was also true for the Copenhagen sample.

In contrast to chronic schizophrenia, the syndromes of latent and probable latent schizophrenia are not limited to the index relatives, but are also found in the biological relatives of adoptees rejected as index or control probands by virtue of a history of psychiatric disorders other than schizophrenia. For example, among the biological relatives of adoptees with a history of major affective illness, 12% (5/30) were found to have latent or probable latent schizophrenia, while only 2% (3/162) of the relatives of control probands received these diagnoses ($p = 0.009$). Taken in conjunction with the finding above of a greater proportion of latent schizophrenia in the relatives of latent schizophrenic adoptees than in relatives of chronic schizophrenic adoptees, this finding raises the question of the specificity the relatives of individuals with schizotypal personality disorder in the current diagnostic nomenclature have to schizophrenia, and whether diagnostic criteria can be developed for a marginal syndrome more specifically related to schizophrenia.

Major affective disorders were not more prevalent in the index relatives than in their controls, confirming the similar observation made in the Copenhagen sample and supporting the genetic distinctiveness of schizophrenia from manic-depressive illness. A significantly higher proportion of the relatives of controls were found to be free of mental illness than any other group of relatives, but neurosis is significantly more common in them (10.5% vs 2.3% in the index relatives, $p = 0.002$), probably reflecting the absence of more serious overriding diagnoses. In general, the only disorders found to be significantly concentrated in the index relatives were chronic and latent schizophrenia.

Counting by probands is the more conservative means of testing the genetic

hypothesis. In this sample 6 chronic schizophrenic adoptees had one or more biological relatives with definite or probable chronic schizophrenia and none among the control adoptees ($p = 0.016$), 10 families with latent schizophrenia and 3 among the controls ($p = 0.045$), and 14 families with chronic or latent schizophrenia compared with 3 among the controls ($p = 0.0034$). Similar but less significant results were found in the Copenhagen study.

A deficiency in the Copenhagen sample was the relative absence of biological full siblings of the probands (there were only 3 in the index and 5 in the control families) so that the significant concentrations of schizophrenic illnesses were found mainly in the biological half-siblings rather than the first degree relatives. In the Provincial sample the bulk of the first degree biological relatives were full siblings, which is attributable to the greater number of stable monogamous relationships existing in the towns and villages, with the result that the first degree relatives in that sample account for most of the significant differences. In the 82 first degree biological relatives of the chronic schizophrenic adoptees compared with the 67 such relatives of the control adoptees there were 7 vs 0 diagnoses of chronic schizophrenia, definite or probable ($p = 0.014$) and 2 vs 0 in the second degree relatives. For chronic and latent schizophrenia, definite or uncertain, the prevalence in first degree relatives was significantly higher than that in their controls ($p = 0.006$) and less strikingly so for the second degree relatives ($P = 0.027$). There is a greater preponderance of schizophrenia and schizophrenia spectrum disorders in the first degree relatives of this sample which is in accord with genetic expectations.

Absence of a concentration of schizophrenia in the index adoptive relatives does not argue against the importance of environmental factors in the etiology of schizophrenia. In addition to the several hundred environmental variables examined in the interviews, efforts were made to obtain specific information relating to current hypotheses. These results are presently under examination and analysis.

Significance to Biomedical Research and to the Program of the Institute

These findings confirm and extend the results obtained previously indicating a strong and quite specific genetic influence in the transmission of classical schizophrenia. They also support a genetic relationship between a milder syndrome - latent schizophrenia of DSM-II or schizotypal personality of DSM-III- and classical schizophrenia, although this milder syndrome is not specific to the relatives of chronic schizophrenic subjects alone. These observations also indicate that the well known tendency of schizophrenia to be concentrated in families is the result of genetic rather than family-associated environmental factors and validate the usefulness of family studies of nonadopted schizophrenics for the examination of genetic influences. A major implication of the operation of genetic factors in etiology is the recognition of the importance of biological, and especially, biochemical factors in this disorder, since the genes can only express themselves through biochemical processes. Another important result of the

more conclusive genetic evidence derived from adoption studies in schizophrenia, already realized, is the rejection of the widely promulgated hypothesis of the 'schizophrenogenic' parent, freeing such parents from an unwarranted and oppressive burden.

Proposed Course

Environmental variables pertaining to socioeconomic class, history of infections and dietary habits, rearing practices, personality of rearing parents, language patterns, communication deviance, expressed emotion, as well as evaluation of cognitive function such as measures of thought disorder, a psychobiological test of smooth pursuit eye movements and one biochemical measure of were included in the new data gathered with the interviews in the Provincial sample. These will be analyzed and correlated with the clinical and demographic data at hand to examine existing hypotheses and possibly to generate some new ones regarding the environmental influences operating in schizophrenia.

The interview data, particularly the large number of items in the mental status examination, will be analyzed to more accurately specify the symptoms and manifestations found among the biological relatives of the chronic schizophrenic probands, with the aim of identifying traits genetically associated with schizophrenia and developing more specific characteristics of syndromes genetically related to schizophrenia in the biological relatives of chronic schizophrenic adoptees. The identification of such a syndrome would aid in the search for clear pedigrees of schizophrenic illness by allowing more individuals to be studied and tested for biological markers than the current low number of biological relatives with frank schizophrenic illness. As part of this search it will be important to differentiate such a syndrome from the milder psychopathology which has been found in the biological relatives of adoptees with other mental disorders such as major affective illness.

A third sample of schizophrenic adoptees has been identified in Denmark, representing adoptees with onset of illness and hospitalization after the previous search through the adoption and psychiatric registers. The biological and adoptive relatives of this sample, with suitable controls, will be examined for mental illness on the basis of hospitalization alone, deferring perhaps indefinitely the expense of the exhaustive psychiatric interviews that have characterized the two previous samples. There is reason to believe on the basis of the results with these samples that in Denmark the number of chronic schizophrenic individuals who never reach a mental hospital is very small.

Publications

Wender, P.H., Kety, S.S., Rosenthal, D., Schulsinger, F., Ortmann, J., and Lunde, I.: Psychiatric disorders in the biological and adoptive families of adopted individuals with affective disorders. Arch. Gen. Psychiatry, 43: 923-929, 1986.

Kety, S.S.: Cerebral circulation and its measurement by inert diffusible tracers. Israel J. Med. Sci., 23: 3-8, 1987.

Kety, S.S.: Neuroscience and psychiatry. Psychiatric Annals, 17: 412-416, 1987.

Kety, S.S. and Matthysse, S. Genetic and biochemical aspects of schizophrenia. In Nicholi, A. (Ed.): Harvard Modern Guide to Psychiatry, Cambridge, MA, Harvard University Press, in press.

Ingraham, L.J., Kety, S.S.: Schizophrenia spectrum disorders. In Handbook of Schizophrenia, Vol. III; M. Tsuang, M., & Simpson, J.C. (Eds.): Amsterdam, Elsevier, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02295-02 I.P.P.

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (60 characters or less. Title must fit on one line between the borders.)

Genetic Factors in Response to Alcohol

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Connie C. Duncan, Ph.D.	Chief, Unit on Psychophysiology	LPP/NIMH
Co-PI:	Frances H. Gabbay, Ph.D.	Guest Researcher	LPP/NIMH
Others:	Allan F. Mirsky, Ph.D.	Chief	LPP/NIMH
	T. Peter Bridge, M.D.	Deputy AIDS Coordinator	ADAMHA

COOPERATING UNITS (if any)

Department of Mental Hygiene, School of Hygiene and Public Health, Johns Hopkins University

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.6

PROFESSIONAL:

1.1

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to assess the relative contributions of genetic and environmental factors to alcohol drinking and response to alcohol challenge. The project is composed of two studies. In the first, 100 male monozygotic (MZ) and 100 male dizygotic (DZ) twin pairs will complete a questionnaire describing their alcohol and other drug use and a 28-day record of their alcohol intake. These data will permit estimation of the relative contributions of genetic and environmental factors to frequency and amount of alcohol consumption. In addition, self-reports of contact between cotwins will permit estimation of the importance of shared environment on cotwin similarities in alcohol drinking. Finally, comparison of the questionnaire estimates of alcohol consumption with those derived from the 28-day record will permit evaluation of the validity of the questionnaire method.

In the second study, 15 male MZ and 15 male DZ twin pairs will receive, in separate testing sessions, a placebo and two doses of alcohol (0.40 and 0.80 g/kg of 95% ethyl alcohol). The protocol will consist of electrophysiological measures (e.g., brainstem auditory evoked responses, resting EEG, and visual and auditory event-related potentials), self-reports of affect, and a measure of standing stability. The use of placebo and multiple doses of alcohol will permit conclusions about the effects of alcohol on information processing, response production, mood, and motor activity. The twin design will provide information on the relative contributions of genetic and environmental factors to variability in these measures in the drug-free state and following response to alcohol challenge. Finally, conclusions regarding the stability of the measures across time will be based on comparisons of baseline measures across the three sessions.

A. Objectives

The primary and most significant objective of this project is to estimate the relative contributions of genetic and environmental factors to alcohol drinking and response to alcohol challenge. In addition, the project will test the effects of alcohol on information processing and response production, on mood, and on motor activity, and examine the stability of alcohol drinking, electrophysiological activity, and response to alcohol across time within individuals. Finally, these data will permit comparison of alternative self-report methods of estimating alcohol intake.

B. Methods Employed

In the first study, 200 male twin pairs (MZ=100 and DZ=100) will complete a comprehensive questionnaire describing their alcohol and drug use. Estimates of monthly alcohol intake will be computed on the basis of these data. The same twins will keep a 28-day record of their alcohol intake. From these "diaries," seven variables describing twins' alcohol use will be computed (e.g., grams of alcohol consumed per weekend day, number of days drinking, maximum amount consumed in a day). Intraclass correlations will be used to assess intrapair similarity, and these estimates will be used to calculate the heritability of alcohol intake (i.e., percent variance accounted for by genetic factors). A self-report measure of "twin closeness" will be taken to estimate the extent to which cotwins interact. This estimate will be compared to estimates of within-pair similarity in alcohol drinking patterns to determine to what extent this component of shared environment accounts for similarity in drinking. Finally, a Pearson correlation will be computed to compare the two methods of estimating alcohol intake.

In the second study, 30 male twin pairs (MZ=15 and DZ=15) will be studied in the laboratory. Over the course of three experimental sessions, they will ingest a placebo, 0.40 g/kg, and 0.80 g/kg 95% ethyl alcohol. The test protocol will include electrophysiological measures (brainstem auditory evoked responses, resting EEG, and visual and auditory event-related potentials), self-reports of affect, and a measure of standing stability. Baseline recordings will be made; and, after the beverage, ERP measurements will be taken once again, while resting EEG, BAERS, standing stability, and the affective measure will be repeated twice. Breath samples will be taken every 10 minutes to estimate blood alcohol levels. Intraclass correlations will be computed to assess intrapair similarity in these measures in the drug-free state and following alcohol challenge. Repeated measures analyses of variance will be used to test the effects of alcohol on these measures. Pearson correlations will be used to assess the relationships among the various measures (e.g., EEG and affect) and to estimate the stability of these measures across time within subjects.

C. Major Findings

Both studies are still in progress. Preliminary results from the first study indicate that MZ twins are strikingly similar in the amount of alcohol

consumed and in their patterns of consumption. Intraclass correlations for the variables derived from the 28-day diaries range from .77 to .91. In contrast, estimates of within-pair similarity for DZ twins indicate that they are, on the average, far less similar than MZ pairs in their patterns of alcohol consumption. This suggests that for males, genetic factors play a role in moderate alcohol consumption patterns, a finding that is consistent with previous research. The pattern of MZ-DZ correlations (e.g., DZ correlations that equal less than half the corresponding MZ correlation) suggests that the genetic contribution is not strictly additive.

Over 40 pairs of twins have been recruited through advertising in the Washington, D.C. Metropolitan area, and these twin pairs are currently being screened for participation in the laboratory study. Computer programs that will permit the recording, analysis, and display of resting EEG, self-reported affect, and standing stability have been developed. Non-twin pilot subjects have been run in the alcohol protocol, and the following results (based on visual inspection of the data, not on statistical analysis) are reported:

1. There appears to be a detectable alcohol effect on the measures we are using. Alcohol caused a slight delay of peaks I through V of the brainstem auditory evoked response (BAER), indicating that transmission time in the brainstem is slowed by alcohol. Further, alcohol appears to cause a decrease in P300 amplitude of the event-related potential and an increase in its latency, suggesting that alcohol also slows cognitive processes which occur later. Spectral analyses of the resting EEG suggests that the EEG is characterized by increased power in the lower frequency range (i.e., theta band) following alcohol ingestion, consistent with the known soporific effects of the drug. Conclusions about the affective change and its relationship to the EEG frequency changes await further analyses.

2. Of great importance is the finding that, while the alcohol effects appear in some subjects, and generally appear in the averaged data, the effects are not equally strong and in the same direction for all subjects. For example, one subject showed a decrease in the latency of BAER peaks. This is consistent with previous findings by the Co-PI, as well as other investigators; but because of statistically significant effects in grouped data, these individual differences typically have not been emphasized in research reports.

3. The electrophysiological measures appear to be very stable within subjects across time. This retest stability has implications for the analysis of alcohol effects and for the estimation of heritability. Within-subject variability increases error variance and decreases the chances of detecting alcohol effects, while retest reliability puts an upper limit on heritability (i.e., cotwin similarity will be limited by the extent to which the trait is stable within a single subject). Thus, their retest stability reinforces the decision to include these measures in this study and suggests they will prove useful in future pharmacogenetic research.

4. Time course effects observed in the early data from this study point

to important methodological issues as well as provide potentially important information on the mechanism of drug action. Recordings were made once before and twice after alcohol ingestion. There were differences across subjects and across variables in the course of effects. In some cases, depressant-like effects occurred immediately, while in others effects were not observed until the second test. As has been reported previously, stimulant-like effects were observed in some cases during the ascending blood alcohol curve, with depressant effects occurring later. Failure to repeat assessment over time would result in incorrect conclusions about the effects, or lack of effects, of alcohol.

D. Significance to Biomedical Research and the Program of the Institute

The significance of the first study lies in its power to estimate the relative contributions of genetics and environment to alcohol drinking in a sample of nonalcoholic males. To date, no such studies based on samples drawn from within the U.S. have been published. Moreover, the use of multiple measures of alcohol intake will increase the confidence in the validity of the findings. Finally, the estimation of social interaction between cotwins and the use of that estimate to account for within-pair similarity in alcohol drinking will provide information on the nature (rather than just the size) of the environmental contributions.

The significance of the second study derives from its use of powerful electrophysiological measures of brain activity within the context of a pharmacogenetic design. This project will shed light on information processing and response production, on disruptions in these processes resulting from alcohol ingestion, and on the heritability of these aspects of brain functioning. Moreover, the finding of individual differences in both baseline electrophysiological characteristics and in response to alcohol will be of great importance in the study of alcoholism. These measures will now need to be employed in studies of individuals at risk for the disorder in order to determine whether these differences are associated with differences in vulnerability to alcoholism.

E. Proposed Course

Data collection for the first study (questionnaires and "diaries") will be completed by the end of 1988. Collection of laboratory data on the twin sample will commence in October and proceed as twin availability allows. Data analysis and preparation of the results for publication and presentation will be conducted as the data are collected.

F. Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02404-01 LPP
PERIOD COVERED July 21, 1987 to September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Psychophysiological Investigations of Preattentional and Attentional Function.		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation) PI: Bruno J. Anthony, Ph.D. Senior Staff Fellow LPP, NIMH		
COOPERATING UNITS (if any) Department of Mental Hygiene, Johns Hopkins School of Hygiene and Public Health; Baltimore City Public Schools.		
LAB/BRANCH Laboratory of Psychology and Psychopathology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>This project applies a new methodology to examine dysfunction in <u>preattentive</u> and <u>attentive</u> mechanisms involved in the regulation of information processing within the central nervous system. Actions of these regulatory mechanisms are assessed through patterns of <u>blink reflex modification</u>, <u>cardio-respiratory change</u> and <u>behavioral measures</u> during simple two-stimulus paradigms. These measures are used to assess preattentive inhibitory and excitatory effects on sensory processing, the integration of different neural systems (sensory, motor and autonomic), and different components of attention including intensity, maintenance, breadth (focus or selectivity), and resistance to distraction. Reports of studies with normal infants, children and adults have been prepared and serve as the groundwork for clinical studies with groups exhibiting different types of regulatory dysfunction. Laboratory facilities and procedures have been developed, protocols have been designed, and pilot work is in its initial phases.</p>		

Project DescriptionA. Other Personnel

Allan F. Mirsky, Ph.D.	Chief	LPP, NIMH
Connie C. Duncan, Ph.D.	Senior Staff Fellow	LPP, NIMH
Sheppard G. Kellam, M.D.	Chairman, Department of of Mental Hygiene	Johns Hopkins University
William W. Eaton, Ph.D.	Associate Professor, Dept. of Mental Hygiene	Johns Hopkins University
Mary Beth Ahearn	Graduate Assistant, Dept. of Mental Hygiene	Johns Hopkins University

B. Objectives

The project is designed to differentiate patterns of regulatory dysfunction among different patient groups by measuring attentive functions and preattentive, automatic processes that underlie adequate attention. The project will be principally concerned with, but not confined to, studies of infants and children. In general, patterns of deficits will be examined in infants characterized as hyperreactive to stimulation, irritable and difficult to calm, children and adults with specific neuropsychiatric disorders (Pervasive Developmental Disorder, Attention Deficit Disorder, Stereotyped Movement Disorders and Anxiety Disorders) or with neurological impairments (seizure disorders, brain damage) as well as those individuals at risk because of familial psychopathology or symptomology related to specific disorders. In addition, groups of infants and children will be followed longitudinally to examine the developmental course of attentive and preattentive deficits, and the relationship between these deficits and the development of neuropsychiatric problems.

C. Major Findings

A major thrust of the proposed work is to study dysfunction in preattentive functions. Organisms have a limited ability to carry out processing that requires attention. Preattentive processes often lay the groundwork for further analysis by attentive processes. As a result, disruption of attention-demanding communicative, social and cognitive activities may be secondary to a distortion of preattentive processing. Such a distortion has been suggested as contributory to a variety of neuropsychiatric disorders; but it has been difficult to assess preattentive processes with traditional, information processing methods. However, the psychophysiological methods employed in the present work--startle blink and autonomic responses--allow for a more direct and powerful way to assess preattentive control of processing. Changes in these measures as a result of stimulation have been shown to reflect the adequacy of important inhibitory (gating), excitatory (arousal), and sensory integrative functions operating without attentional or conscious control.

A summary of the work supporting the usefulness of the measures in examining preattentive processes across the developmental span will appear in a forthcoming volume of Advances in Infancy Research. Also, a report of previous studies with normal volunteers detailing the surprisingly uneven development during childhood of preattentive, inhibitory processes was prepared and submitted for publication. Another report of a series of studies utilizing the blink reflex to document important developmental changes in the processing of brief or transient stimuli was published this year. Investigations of the effects of temporal characteristics of stimulation on responsiveness deserves systematic investigation because problems in the processing of transients appear to be involved in perceptual difficulties of the aged, of dyslexic and dysphasic children, of autistic children, and in the dysfunction following lesions of sensory cortex.

The psychophysiological measures employed in this project are also sensitive to attentive processes and, in conjunction with behavioral measures, can be used to distinguish different components of attention. We are currently preparing chapters summarizing a series of previous studies with infants, children and adults which investigated the ability to mobilize attentional resources, distribute these resources selectively and to resist distraction. These chapters will appear in a book that will integrate central and autonomic nervous system approaches to the study of human information processing.

In the first few months of work on this protocol, we have revised and developed computer programs for stimulus delivery and timing as well as data collection. In addition we have purchased, installed and tested additional equipment for the measurement of autonomic and skeletal muscle responses as well as the more precise control of acoustic and visual stimulation. We are currently involved in pilot work with normal volunteers to examine the quality of the data and to set stimulus parameters for work with clinical populations.

A major part of this project will be the longitudinal examination of children attending second and third grade in the Baltimore City Public Schools who will be selected on the basis of their performance on a battery of neuropsychological tests. The neuropsychological battery was administered to a representative sample of 435 children in the Spring of 1987. In the past few months we have begun the examination of these data, and preliminary factor analysis has revealed distinguishable components of attentive functioning. Beginning in the Fall of 1987, a group of children deemed at highest risk from this battery and a representative sample of the population who do not appear at risk will be brought to the LPP to participate in this project. The goal of this study is to examine and describe neurobiological indices of attention, and of the processes that underly attention and, further, to examine their relation to behavioral and neuropsychological indices. Moreover, we hope to follow these children longitudinally to examine the stability of these psychophysiological indices and their usefulness as predictors of later maladaptive behavior.

Significance to Biomedical Research and to the Program of the Institute

Many children who do poorly in school and/or exhibit learning difficulties or significant neuropsychiatric disturbances appear to have attentional deficits. In addition, the severity of learning problems increases as the child grows older. Attention problems are also associated with behavioral disturbance in childhood. One-half of the referrals to the nation's mental health clinics are for attention-related problems. Also, it is becoming increasingly clear that attention disorders can no longer be considered only as problems of childhood. One quarter to one half of adolescents with Attention Deficit Disorder are referred to the courts for theft and truancy and one quarter are later diagnosed as Anti-social Personality Disorder. Moreover, attention problems may represent a factor which increases the vulnerability of those children already at genetic risk for serious psychopathology such as schizophrenia. This work contributes to an understanding of the neurobiologic dysfunction underlying attention disorders, to the separation of more global "attentiveness" into different components, and to the role of attention problems in the development of neuropsychiatric problems.

Proposed Course

Besides the longitudinal examination of 2nd and 3rd graders outlined above, we plan to embark on two other studies. The first will compare a sample of normal boys with three groups of boys each of which exhibits different behavioral patterns of regulatory dysfunction: Attention Deficit Disorder with Hyperactivity, anxious-inhibited behavior and Tourette's Syndrome. The second study will examine groups of 4-month-old infants at risk for the development of regulatory problems because of familial attention disorders or because of temperament features. The latter group will consist of infants characterized as overly active, easily agitated, difficult to calm, and hyperreactive to stimulation. Basic aspects of regulation, such as inhibition, arousal, orientation and attention are relevant to an integrated transaction between the infant and the environment, and difficulties with these functions may place the infant at risk. As the potential for early intervention increases, it becomes more important to evaluate children as early as possible with an eye to preventative and therapeutic approaches. The infant studies will examine neurobiological indices of dysregulation, the stability of these indices, and their predictive validity for later development of neuropsychiatric difficulties.

Publications

Anthony, B. J., Zeigler, B. L., & Graham, F. K.: Stimulus duration as an age-dependent factor in reflex blinking. Dev. Psychobiol., 20: 285-297, 1987.

Anthony, B. J., Graham, F.K., & Balaban, M.T.: Mechanisms of selective processing in development. In Rovee-Collier, C. (Ed.): Advances in Infancy Research. In press.

Balaban, M. T., Anthony, B. J., & Graham, F. K.: Prestimulation effects on blink and cardiac reflexes of 15-month human infants. Dev. Psychobiol. In press.

Anthony, B. J.: Developmental changes in information processing. In Jennings, J. R. & Coles, M. G. H. (Eds.): Psychophysiology of information processing: An integration of central and autonomic nervous system approaches. Sussex, England: John Wiley. In press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00672-22 LSES

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Social Psychological Correlates of Occupational Position

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Schooler, Acting Chief, Laboratory of Socio-environmental Studies, NIMH

OTHER: C. Schoenbach
M. KohnSocial Science Analyst
Guest ResearcherLSES
LSESNIMH
NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

5.00

PROFESSIONAL:

.50

OTHER:

4.50

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The object of this study is to assess the reciprocal effects of occupational conditions and psychological functioning (in particular, values, self-conceptions, social orientation, and intellectual flexibility). Structured interviews were conducted in 1964 with a sample of 3101 men, representative of all men employed in civilian occupations throughout the United States. The study was extended into a longitudinal study in 1974, with the reinterviewing of a randomly-selected one-fourth of the original sample, together with their wives and, where appropriate, one of their children. Replications of this research have been carried out in Poland and Japan.

Project Description:

The central aim of this study is to examine how the psychological effects of the different occupational conditions faced by people in various positions in the social hierarchy help explain the differences found in their psychological functioning. It began in 1964 with structured interviews with a sample of 3100 men, representative of all men employed in civilian occupations throughout the United States. In 1974 follow-up interviews were conducted with a randomly selected one fourth of the original respondents, as well as with their wives and with one of their children.

The central findings have been that self-directed work leads to intellectual flexibility and to a self-directed orientation to self and society and that oppressive working conditions lead to distress. These findings have been found to hold true under a variety of different conditions (paid employment, school work, women's housework) and at different stages in the life span. They have also been replicated in separate studies carried out by the Laboratory and its collaborators in Poland and Japan.

The most important accomplishment this year has been the completion of a major paper directly comparing the effects of position in the social class structure on psychological functioning in the United States, Japan, and Poland. This work was carried out by Melvin Kohn, presently a guest researcher in the Laboratory, and Carrie Schoenbach and Carmi Schooler, together with Atsushi Naoi of the University of Osaka and Kazimierz Slomczynski of the University of Warsaw, both former Visiting Scientists in the Laboratory. For each of the three disparate countries, social classes were conceptualized and indexed in terms of ownership and control of the means of production and control over the labor power of others. This was done in ways that took into account the particular historical, cultural, economic, and political circumstances of each country. We found, as we had hypothesized, that men who are more advantageously located in the class structure of their society would be more likely to value self-direction in their children, to be intellectually flexible and to be self-directed in their orientations than are men who are less advantageously located. We also found, as we had also hypothesized, that occupational self-direction plays a crucial role in explaining the psychological impact of social class in each of these quite disparate countries.

We also uncovered relationships between social class and a sense of distress. Their pattern, however, differed by country: In the United States, managers have a strong sense of well-being and manual workers are distressed; in Poland, just the opposite; and in Japan managers have a strong sense of well-being, but it is the white collar workers, not the blue collar workers, who are most distressed. Both a self-directed, intellectually flexible mode of psychological functioning and a sense of distress can affect the individual's mental health. Both can be affected by social structure and culture. Our findings indicate that although the

way social structure affects psychological self-directedness and flexibility is generally the same in quite different countries, the determinants of distress can be a function of country-specific cultural and historical factors.

Other analyses on the effects of social class that were done this year were those by Schooler and Schoenbach examining the relationship between income and social class in the three countries. A central finding is that class has a greater affect on income in communist Poland than it does in the two capitalist countries.

This year also saw the continued analysis of the thorny problem of isolating the effects of parental behavior in the familial transmission of various modes of psychological functioning. Also examined, was the question of how traditional culture and social conditions interact to affect Japanese women's feelings towards the interdependence of family members. This was explored by examining how these factors affect their feelings of responsibility for taking their own and their husbands' aged parents into their households. Taking care of the elderly in this manner is seen in traditional Japanese culture as a central duty. Our initial analyses suggest that not only do such attitudes continue to exist, but also that they are correlated with the woman's working in a traditional industry, doing relatively simple and nonself directed work both on the job and at home, and coming from a rural and lower social economic status background, as well as with having a husband with the same general social characteristics. It also appears that these relationships are even stronger for the women's beliefs about their childrens' responsibilities towards taking them in when they themselves are aged. This suggests that, at least in the Japanese context, change may be occurring more in terms of wanting increased independence for oneself when one is older, than in rejecting traditional norms about one's responsibilities towards one's own elders.

Significance of the Research:

Because the characteristics of both more and less complex levels of phenomena affect intermediate ones, to understand human psychological functioning we have to have a basic knowledge of both the human central nervous system and the human social system. Recognizing the importance of the social level of phenomena in determining human behavior means that the study of the human social system cannot be totally neglected without seriously imperiling the search for the root causes of psychological dysfunction. Although the examination of social level phenomena thus represents a legitimate basic research goal, the LSES, in light of its limited resources, has focused on those aspects of the social environment which we have reason to believe most directly affect psychological functioning. Where we have studied social level phenomena in themselves, it has been with the aim of knowing more about those aspects of the social environment that may affect people psychologically. In fact, almost all of our research has been concerned with the causal connections between the

social and cultural systems and the individual's psychological functioning. In particular, we have focused on how individuals' positions in the social system affect their cognitive functioning, interrelationships with others and ability to cope with life's stresses. These three areas, besides being essential for our basic understanding of human nature, are clearly implicated in mental illness. Schizophrenia, for example, is characterized by cognitive dysfunction, social withdrawal and an inability to cope with social and other forms of stress. In addition, direct evidence of the relevance of social level phenomena to schizophrenia can be found in the fact that for reasons that are still not clear, there is a negative relationship between social class and the incidence of schizophrenia.

Recent, carefully controlled research suggests that noisome, dangerous, and uncomfortable occupational conditions, which are more likely to be characteristic of lower status jobs, play a part in the development of schizophrenia. Research carried out by the Laboratory indicates that people at lower stratification levels have less effective cognitive and other psychological mechanisms for coping with stress and uncertainty and more rigid and conformist orientations towards others. Thus oppressive job conditions, cognitive and other forms of coping mechanisms, and the nature of the individual's orientation to others are linked to social status in ways that suggest that they may play a part in the etiology of schizophrenia. If the problem of schizophrenia is to be truly faced, basic socio-environmental research on these problems must accompany basic research on the molecular and neurological level.

Beyond NIMH's concern with mental disorder is the Institutes mandate to study the conditions that facilitate and those that interfere with effective psychological functioning. This research demonstrates that job conditions have appreciable effects on cognitive performance, self conceptions and orientations to the outside world and thus are directly linked to the mental health and effective psychological functioning of the individual.

Proposed course of further research:

Data has been collected on the psychological effects of occupational and other social structural conditions on a sample of Japanese fathers, mothers and children that parallels the data we have collected in the United States. The analyses of the data on the Japanese men have already been completed and reported. We now plan to analyze the women's and childrens data to investigate the interaction of culture and social structure on the individual's intellectual and interpersonal functioning. This Japanese data will also be used to examine the nature of the intrafamilial transmission of psychological functioning, and of orientations to self and society. The results of these analyses will serve as a basis of comparison with those of similar, presently ongoing analyses of our American data. Such comparisons will permit us to assess the cross-cultural generalizability of our results as well as the way in which culture interacts with social structure and parental influence to affect the psychological functioning of the individual.

Publications:

Kohn, M.L.: Unresolved Issues in the Relationship between Work and Personality. In Kai Erikson (Ed.): Working. New Haven: Yale University Press, in press.

Kohn, M.L., and Schoenbach, C.: Social Stratification and Parental Values: A Multi-national Assessment. In Tominaga, K. and Treiman, D. (Eds.): Social Stratification and Mobility in Japan and the United States. Connecticut, Greenwood Press, in press.

Kohn, M.L., and Schooler, C.: Shigoto to Personality. (Work and Personality.) Tokyo, Japan: Saiensu-sha Publishing Co., 1987, in press.

Miller, Karen A., Kohn, M.L. and Schooler, C.: Educational self-direction and personality, Am. Soc. Rev., 5: 372-390, 1987.

Miller, K.A., and Kohn, M.L.: The Reciprocal Effects of Job Conditions and the Intellectuality of Leisure-time Activity. In Tominaga, K. and Treiman, D. (Eds.): Social Stratification and Mobility in Japan and the United States. Connecticut, Greenwood Press, in press.

Roberts, B.: A Confirmatory Factor-analysis Model of Alienation. Soc. Psych. Q., in press.

Schooler, C.: Psychological and Social Perspectives on Status Attainment. In Tominaga, K. and Treiman, D. (Eds.): Social Stratification and Mobility in Japan and the United States. Connecticut, Greenwood Press, in press.

Schooler, C. and Naoi, A.: The Psychological Effects of Traditional and of Economically Peripheral Job Settings in Japan. Am. J. Sociol., in press.

Schooler, C.: Using Linear Structural Equations Analysis to Estimate the Reciprocal Effects of Job Complexity and Ideational Flexibility. J. Voc. Beh., 31: 1987, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00679-07 LSES

PERIOD COVERED

October 1, 1986 through September 30, 1986

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Equation Models in the Analysis of Data with Measurement Error

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ronald J. Schoenberg, Research Sociologist LSES NIMH

OTHER: C. Schooler Acting Chief LSES NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

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SECTION

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.10

PROFESSIONAL:

1.10

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

The purpose of this work is to further develop the methods and techniques for the specification and estimation of the parameters of structural equation models of survey data that contain random and nonrandom measurement error. Included in this are methods for the identification of the models, estimation of the means of unobserved variables, the determination of model condition, and the treatment of polytomous variables.

Project Description:

Diagnosis is an important medical problem. It amounts to a problem in the assignment of people to classes or categories on the basis of symptoms. This assignment can be accomplished quite easily if the pathology as well as its physiological relationship with the symptoms, are well understood. But in some cases neither the disease nor its relationship to symptoms is very well understood. This is the case with schizophrenia.

Drawing on the statistical literature on "latent class analysis" Ronald Schoenberg and Carmi Schooler are developing statistical models of the relationship of schizophrenia to symptoms. These models are similar to "cluster models", which are well developed in psychometric research, but were not designed for categorical or discrete variables which is the form of most measures of symptoms.

Latent class models can be used in an exploratory way to assist in discovering first, the dimensionality of the underlying latent constructs, in this case, the dimensionality of schizophrenia, and second, the relationships of the latent constructs to the observed measures or symptoms. And when a specification has been decided upon then the latent class analysis can be used to produce a method for the classifying individuals into latent categories, that is, to diagnose.

The method of latent class analysis is being applied to items in the Diagnostic Interview Schedule (DIS) data in the Epidemiological Catchment Area study conducted for NIMH that purport to measure schizophrenia. Early results of this analysis supports a multidimensional explanation for the DIS symptoms.

A long recognized problem in non-experimental research at NIMH has been the issue of sampling. Most subjects of studies at NIMH are found through referrals from clinics, hospitals, etc., or are volunteers. And the subjects actually studied must pass through a stringent qualifying process that usually excludes people on medication or with chronic diseases. This produces two problems: first, a problem in generalization, and second, a problem in determining the outcome without bias.

The first problem is generally well known to researchers but the second is not so well known. If the factors that determine whether a subject will be included in the study are related in some way to the dependent variable in the study, then the usual methods for calculating the outcome-- multiple regression, for example--will produce biased estimates of the outcome. Thus sample selection can attenuate or even reverse findings. Solving the second problem, then, can be critical for many nonexperimental studies that do not randomly sample from a population.

The solution for both of these problems requires that models be constructed for the sample selection process. That is, models--in the simplest case regression models--must be developed that predict the probability that a subject with given characteristics will be included in the study. Adjustments then can be made in the analysis that will remove the bias in estimating the outcome due to the nonrandom sample selection, and permit generalization of the outcome to the general population.

Ronald Schoenberg, using data from the Epidemiological Catchment Area study, is developing models for predicting the use of mental health facilities which is the first step a subject must take to become included in a study at NIMH. This model will then be used as a part of a further analysis of the adjustments that can be made to studies conducted at NIMH to correct for sample selection bias and to permit generalization to the general population.

Significance of the Research:

The development of the latent class models of schizophrenia has helped to establish the multidimensionality of schizophrenia. The specification of a measurement model for schizophrenia has implications for the diagnosis of schizophrenia--it will now be possible to provide a rigorous method for diagnosis on the basis of observed symptom. This model may also provide some clues for further investigation into the etiology of schizophrenia.

Proposed Course of Further Research:

The measurement model of schizophrenia will be replicated in other data sets to test its validity. The sample selection models will be further developed and tested on clinical sample data to determine their usefulness in enhancing generalization and undoing the effects of sample selection bias.

References:

- Schooler, C.: A Statistical Interaction Approach to Problems in Cross-Cultural Epidemiology. To be published in the proceedings of the International Symposim on Psychiatric Epidemiology, Taipei, Taiwan, in press.
- Schooler, C.: On Levels of Research: The Prospective of a Psychologist Practicing Psychology. In Matilda Riley (Ed.): Social Structures and Human Lives. 1986. Presidential Papers, American Sociological Association, Washington, D.C., in press.
- Zahn, T.P., Schooler, C., and Murphy, D.L. Autonomic Correlates of Sensation Seeking and Monoamine Oxidase Activity: Using Confirmatory Factor Analysis on Psychophysiological Data. Psychophysiology, 23: 521-531, 1986.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00680-05 LSES

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Work Experiences and the Deinstitutionalized Mentally Ill

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Elliot Liebow, Guest Researcher, LSES, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objective of this exploratory, participant observation study is to examine the work experience of the deinstitutionalized mentally ill over time and to seek out ways in which job characteristics, symptoms, and social relationships interact with one another to effect the course of recovery from psychiatric disorder and reintegration into the community. Field work was carried out with residents of halfway houses, participants in community-based psychosocial and transitional work programs, and with "unattached" deinstitutionalized men and women.

Project Description:

Four years ago, Elliot Liebow, on detail to the Laboratory from the Extramural Program, began an exploratory, participant-observer study of the relationship between work experience and recovery from mental illness. The goal of this research was not to test hypotheses but rather to grasp the dynamics of the interaction between work experiences and recovery from mental illness.

In 1984, while still collecting data on deinstitutionalized persons in halfway houses and psychosocial programs in Montgomery County, Md., Liebow was stricken by two successive major illnesses. He retired on disability in September of that year but remained as a guest researcher in order to try to salvage some of the data he had already collected. These were somewhat too thin to serve their original purposes but were potentially useful nonetheless.

In November of 1984, Liebow began collecting data as a participant observer in a shelter for homeless women in Rockville. Many of the two dozen women who are "regulars" (in the sense that they stay at the shelter night after night, month after month) as well as the more casual users who come for a night or two, have a history of mental illness and/or institutionalization. Through these participant observation experiences, Liebow plans to contrast the dynamics and outcomes of two post-institutionalization life-styles: (a) the highly structured, tightly supervised group living of halfway houses and psycho-social day programs versus (b) the relatively unstructured, free floating life style of shelters and soup kitchens.

This research will focus primarily on the two dozen women who are "regulars" at the shelter. Liebow has now followed them intensely for more than 24 months. Data collection is completed, coding categories have been developed, and analysis and writing are underway.

Significance of the Research:

This project is directly pertinent to our understanding of rehabilitation of the deinstitutionalized mentally ill.

Proposed Course of Research:

Formal data collection has been completed. The investigator is working on the analysis and writing up of this data as a Guest Researcher at a moderate pace.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00681-01 LSES

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Reciprocal Effects of Self-Esteem and Depression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Rosenberg, Guest Research, Laboratory of Socio-environmental Studies, NIMH

OTHER:	C. Schooler	Acting Chief	LSES	NIMH
	C. Schoenbach	Social Science Analyst	LSES	NIMH

COOPERATING UNITS (if any)

None

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Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.05

PROFESSIONAL:

.55

OTHER:

.50

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Although low self-esteem has been shown to be associated with a number of non-psychotic disorders among children, adolescents, and adults, the causal connection among these variables has not been established. In order to do so, we plan to make use of a longitudinal data set. This is the Youth in Transition study, a five-wave national probability sample of adolescents. Through the use of instrumental variables and structural equation models, we will seek to specify the reciprocal effects of global self-esteem and a number of mental health and behavioral variables.

Project Description:

The aim of this project is to explore the reciprocal effects of global self-esteem and selected mental health and behavioral measures. Past research dealing with the relationship between self-esteem and other variables has been unable to answer the question: Which is cause and what is effect? For example, although many studies show a strong relationship between global self-esteem and depression, we do not know whether self-esteem is responsible for depression or depression is responsible for self-esteem. To be more exact, we do not know the extent to which each variable affects the other. The same basic question can be asked of the relationship between self-esteem and many other important mental health and behavioral variables.

Our purpose is to explore such questions by means of reciprocal effects analyses. In order to do so, we plan to make use of a large scale longitudinal data set containing a wide range of relevant variables. This is the Youth in Transition study, a five-wave national probability sample of 2213 adolescents. The use of such longitudinal data enhances the possibility that structural equation models can be developed that will enable us to specify the reciprocal effects of these variables. Dr. Schoenberg, an expert in structural equation modeling, is available to help us solve the complex statistical problems that such analysis entails.

Significance of the Project:

The relevance of global self-esteem for mental health has been repeatedly demonstrated in the literature, and is supported both by survey and experimental data. Studies conducted with children, adolescents, and adults show that low self-esteem is associated with depression, somatic symptoms of anxiety, psychological anxiety, irritability, loneliness, resentment, hypersensitivity, external locus of control, and low life satisfaction. Low self-esteem is also associated with interpersonal inhibition, apathy, withdrawal, and other behavioral difficulties. What is unknown is the reciprocal effects of self-esteem and these other variables. Such findings have implications for therapeutic strategies. They can suggest, for example, whether it is advisable to attempt to deal with depression by altering self-esteem or whether, for this purpose, this strategy is a waste of effort. Similar questions can be asked with respect to many other correlates of self-esteem.

Proposed Course of Project:

Initial problems of coding and data organization have now been solved and a workable data tape is available. The reciprocal effects analyses are now in process.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00682-01 LSES

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Environmental Determinants of Cognitive Functioning

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: L. Caplan, Staff Fellow

LSES

NIMH

OTHER: C. Schooler, Acting Chief

LSES

NIMH

COOPERATING UNITS (if any)

None

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TOTAL MAN-YEARS:

1.60

PROFESSIONAL:

1.35

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

 (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is to investigate the effects of task complexity on cognitive functioning. Subjects were trained to use a computer program under different conditions of complexity. After training, the following were assessed: 1) knowledge structure development, 2) flexibility of problem solving, and 3) planning ability.

Project Description:

Over the last 20 years, Melvin Kohn and Carmi Schooler have published a series of studies which have demonstrated that environmental complexity (e.g., complexity of occupation, schoolwork, housework) encourages the development of intellectual flexibility. The results have been reliably demonstrated in a number of different populations: American men, American women, Japanese men, Polish men, American schoolchildren.

Such effects are almost certainly the result of cognitive development which occurs as individuals attempt to deal effectively with their environments (see Schooler, 1984). Until recently, however, the exact nature of the cognitive changes involved has not been examined. The project described here is designed to test a number of hypotheses which may account for the intellectual flexibility results obtained in earlier studies. In particular, the experiment described below was designed to test the following hypotheses about the effects of environmental complexity:

1. Environmental complexity encourages the development of highly detailed and elaborated knowledge structures, which in turn enable an individual to engage in flexible problem solving.
2. Environmental complexity encourages the development of metacognitive planning skills, i.e., those skills which enable individuals to plan and regulate their own cognitive activities.
3. Environmental complexity is most likely to facilitate the development of flexible problem solving when it occurs in a task which is inherently meaningful.

The experiment involved training people in the use of a microcomputer drawing program - a task similar to those they encounter in daily life. The training conditions involved manipulating: 1) the degree to which training materials were organized, 2) whether or not a concrete model of the program's functioning was provided, 3) the degree of visual complexity of the stimuli, and 4) the degree of decisional complexity required by the task. Following training, tests were administered which assessed subjects' ability to engage in flexible problem solving with the program, and the development of mental representations of the program.

Data from the complete sample of 128 individuals have been collected. In addition to data from the experimental task described above, we have also collected demographic information, information about occupational

complexity, and scores from two subtests of the Wechsler Adult Intelligence Scale from each subject.

Significance of the Research:

The results of this study should provide some initial explanations of the effects of environmental complexity previously demonstrated in this laboratory. If, in fact, individuals exposed to the complex training provided in this study develop more elaborated mental representations of the program, or more advanced metacognitive planning abilities, then these results will support the hypothesis that increased environmental complexity facilitates the development of qualitatively different cognitive abilities than those developed in response to simpler environmental demands. Such findings would both explain the earlier results from Kohn & Schooler data, and open up new avenues for research on the specific cognitive effects of environmental complexity.

In addition, the results of this study will be a significant contribution to the literature on human problem solving in cognitive psychology. Much of this literature has focused on relatively simple problems, with well-defined questions and clear solutions. The type of problem-solving required by this task, as by many problems encountered in everyday life, involves learning to deal with problems which are not well-defined. The results of this project, therefore, should provide cognitive psychology with a description of the problem-solving processes involved in more ecologically valid domains.

In related work, Schooler is involved in the organization of a series of conferences aimed at linking cognitive psychology, to the study of the effects of social structure on individual functioning over the life course.

Proposed Course of Research:

The next step of this research project involves data analysis and writing the results up for publication.

Publications:

Schooler, C. and Schaie, (Eds.): Cognitive Functioning and Social Structures over the Life Course. New Jersey, Ablex Publishing Co. 1987.

Schaie, K. W. , and Schooler, C. (Eds.): Social Structure and Aging: Psychological Processes. Hillsdale, New Jersey, Erlbaum, in press.

Schooler, C.: Social Structural Effects and Experimental Situations: Mutual Lessons of Cognitive and Social Science. In Schaie, K. W. and Schooler, C. (Eds.): Social Structure and Aging. Hillsdale, New Jersey, Erlbaum, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00424-12 LCB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biologically Active Peptides in the Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.s

Michael J. Brownstein, Chief, Laboratory of Cell Biology, NIMH
T. Bonner, Special Expert, Laboratory of Cell Biology, NIMH
C. Gerfen, Sr. Staff Fellow, Laboratory of Cell Biology, NIMH
M. Palkovits, Visiting Scientist, Laboratory of Cell Biology, NIMH
W. S. Young, Sr. Staff Fellow, Laboratory of Cell Biology, NIMH
(see attached)

COOPERATING UNITS (if any) Univ. Zuerich; Tulane Univ; Neurol.Sci.Inst., Portland;
MD NIDDKD; CNG NIMH; BG NHLBI; ERR CH; Tufts U. Med. Sch; Westminster Hosp.,
London; LVC NCI; LMG NINCDS; BPB NIMH; Karolinska Inst; LNC NINCDS

LAB/BRANCH

Laboratory of Cell Biology

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TOTAL MAN-YEARS:

10

PROFESSIONAL:

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have continued to study the organization and development of peptidergic and non-peptidergic neurons in the central nervous system and periphery, biosynthesis of biologically active peptides, factors that regulate peptide secretion, and receptors and their second messenger systems.

Other Professional Personnel Engaged on Project

H.-U. Affolter	Ober Assistant	Univ. Zuerich
A. Arimura	Professor	Tulane Univ.
N. Barmack	Sr. Scientist	Neurol.Sci.Inst., Portland, OR
W. Benson	Biologist	LCB, NIMH
M. Brann	Staff Fellow	MD, NIDDKD
N. Buckley	Visiting Fellow	LCB, NIMH
J.-M. Burgunder	Guest Researcher	LCB, NIMH
S. Detera	Sr. Staff Fellow	CNG, NIMH
T. Gorcs	Scientist	Tulane Univ.
J. Gutierrez	Chemist	LCB, NIMH
D. Hilt	Sr. Staff Fellow	BG, NHLBI
S. Horvath	Visiting Fellow	LCB, NIMH
L.-S. Hsieh	Visiting Fellow	BG, NHLBI
F. Huang	Staff Fellow	ERR, CH
K.-P. Huang	Section Chief	ERR, CH
C. Jelsema	Guest Researcher	LCB, NIMH
J. King	Professor	Tufts U. Med. Sch.
D. Kligman	Staff Fellow	LCB, NIMH
K. Koller	Staff Fellow	LCB, NIMH
M. Konig	Microbiologist	LCB, NIMH
R. Lad	Visiting Fellow	LCB, NIMH
R. Lee	Guest Researcher	LCB, NIMH
S. Lightman	Reader in Medicine	Westminster Hosp., London
S. Lolait	Guest Researcher	LCB, NIMH
L. Mahan	Staff Fellow	LCB, NIMH
L. Matsuda	Guest Researcher	LCB, NIMH
E. Mezey	Visiting Scientist	LCB, NIMH
W. Modi	Geneticist	LVC, NCI
J.-M. Muller	Visiting Fellow	LCB, NIMH
S. O'Brien	Acting Laboratory Chief	LVC, NCI
H. Okayama	Visiting Scientist	LCB, NIMH
P. Padgett	Chemist	LMG, NINCDS
J. Patel	Visiting Associate	BPB, NIMH
A. Rokaeus	Asst. Professor	Karolinska Inst.
E. Shepard	Microbiologist	LCB, NIMH
M. Warden	Biologist	LCB, NIMH
S. Wray	Sr. Staff Fellow	LNC, NINCDS
R. Wolff	Biol. Lab. Tech.	LCB, NIMH
A. Young	Chemist	LCB, NIMH
R.T. Zoeller	NRC Fellow	LNC, NINCDS

Project Description

Peptide/protein isolation and characterization

Drs. Kligman and Patel have purified a major 87kD substrate of C kinase from brain extracts and, with the help of J. Gutierrez, have the amino acid sequences of several tryptic peptides derived from this protein. In collaboration with S. Detera, they are attempting to clone a cDNA that encodes this protein.

Cloning and sequencing of cDNAs and genomic DNAs

T. Bonner, A. Young, N. Buckley, and M. Brann have shown that there are at least four distinct muscarinic receptors by isolating, sequencing, and expressing the DNAs that encode them. Three of the receptors are of the M1 type, one is M2. The receptors have unique distributions in the CNS suggesting that they subserve different functions.

Drs. Bonner, O'Brien, and Modi are mapping the chromosomal location of the four muscarinic receptor genes isolated to date, and Drs. Bonner and Detera are looking for restriction fragment polymorphisms to use to determine whether the receptor genes are linked to mental illness.

T. Bonner, U. Affolter, and A. Young have cloned a rat cDNA encoding the precursor of neurokinin B (neuromedin K) and characterized its mRNA in rat brain. Dr. Bonner has also cloned and completely sequenced the human neurokinin B gene and has shown that two upstream exons proposed to be part of the gene are the last two exons of an adjacent gene.

Drs. Bonner, O'Brien, and Modi have mapped the chromosomal location of the human substance P and neurokinin B genes to 7q 21.1-22 and 12q 13.1-21.1, respectively.

Drs. Kligman and Hilt have cloned a full length cDNA that encodes S100 β , a neurite extension factor.

Dr. Koller isolated a cDNA that encodes the valocin "precursor." She has shown that this protein is unlikely to give rise to a peptide secretory product; it is found in the cytoplasm of diverse cells. This is the first example of the use of molecular genetics to eliminate a peptide as a transmitter candidate.

Dr. Mezey has cloned the cDNA that encodes the rat phenylethanolamine-N-methyltransferase so that she can construct oligonucleotide probes and raise antibodies to use for in situ hybridization histochemistry and immunocytochemistry.

Studies of receptors

We are currently exploring several strategies for cloning cDNAs that encode receptors. Since J. Gutierrez and P. Padgett are capable of microsequencing peptide fragments, we could, in principle, purify and partially sequence receptor proteins. We have thus far elected not to do this. Instead, we have chosen to concentrate on eukaryotic expression cloning and low stringency hybridization with oligonucleotide probes based on highly conserved domains of characterized receptors. The latter strategy has already proven quite fruitful (see the section above).

Dr. Buckley has developed a method for transferring colonies of mammalian cells to polyester sheets. The "replicas" can be screened for the presence of cells that are expressing receptors of interest by means of receptor autoradiography.

number of methods have been developed for enriching libraries for cDNAs encoding receptors including size-selection and screening with transient expression assays. Collaborations have been established that will allow us to screen libraries by injecting mRNA derived from our cDNAs into *Xenopus* oocytes. Very big libraries with especially large inserts are being prepared in a novel vector for this purpose. Finally, recent improvements in transfection methodology (see Dr. Okayama's section) promise to contribute significantly to our ability to produce large eukaryotic expression libraries.

At present Drs. Buckley, Lolait, Muller, Matsuda, Mahan, Rokaeus, Young, and Koller are endeavoring to isolate receptor cDNAs including those for substance P, angiotensin, VIP, bradykinin, somatostatin, galanin, neurotensin, and CCK.

Dr. Young has recently isolated the gene for the rat neural nicotinic receptor and is testing the hypothesis that it undergoes a novel alternative splicing in hypothalamic neurons.

Functional neuroanatomy

Drs. Young, Burgunder, Koller, Zoeller, Lightman, Mezey, Gerfen, Buckley, and Lolait have continued to improve and exploit the ISHH method.

Drs. Young and Burgunder are using ISHH to study factors that regulate vasopressin and oxytocin mRNA levels in the supraoptic nucleus.

M. Warden and her co-workers have mapped tachykinin mRNAs in the CNS.

S. Young and S. Lightman have studied the regulation of hypothalamic enkephalin mRNA levels.

S. Young and N. Barmack have used ISHH to examine the role of inferior olivary CRF-cells in regulating eye movements.

Drs. S. Young, K.-P. Huang, and F. Huang have studied the distribution and development of protein kinase C.

K. Koller, T. Zoeller, and R. Wolff have shown that thyroid hormones influence the levels of TRH message and that TSH is not required for these hormones to exert their effects on TRH mRNA.

T. Zoeller, S. Wray, and J. King have examined the role of estrogen in controlling GnRH biosynthesis.

Dr. Buckley and his colleagues have used ISHH to map muscarinic receptor subtypes in the CNS and in peripheral tissues.

Dr. Gerfen has demonstrated that pharmacological manipulation of the dopaminergic mesostriatal system differentially regulates patch-matrix neuronal expression of neuropeptides.

Dr. Lolait has used ISHH to look for neuropeptide mRNAs in peripheral tissues.

Immunocytochemistry

S. Horvath, M. Palkovits, T. Gorcs, and A. Arimura have shown reciprocal connections between GRF- and somatostatin-producing neurons in the hypothalamus.

L. Mahan, C. Jelsema and M. Palkovits have used immunocytochemistry to examine the nature and distribution of G proteins in the retina and to study the role of somatostatin in regulating the circadian rhythm in indoleamine metabolism in the retina of *X. laevis*.

Dr. Gerfen and his colleagues have examined the organization and development of the basal ganglia by means of neuroanatomical and neurochemical techniques. Their previous work showed that the striatal patch-matrix organization reflects the existence of parallel input-output systems which connect the cerebral cortex through the striatum to the substantia nigra. Current work demonstrated the compartmental organization of mesostriatal dopaminergic systems. Furthermore, as the matrix striatonigral system expresses the brain calcium binding protein calbindin-D28kD it was also shown that this protein also distinguishes the subset of dopaminergic neurons projecting to the matrix. 3H-thymidine labeling showed that striatal patch neurons are born prior to matrix neurons. The asynchronous migration of these neurons along immunohistochemically labeled radial glia show the manner in which the patch-matrix organization develops. Studies directly demonstrated the transformation of striatal radial glia into astrocytes. Following neuronal migration this

transformation process occurs to establish neuron-glia relationships in the patches prior to the matrix.

Significance to Biomedical Research

Nerve cells use chemical "transmitters" to communicate with one another and with other target cells. Changes in transmitter biosynthesis, release, and/or metabolism have been suggested to result in nervous and mental disorders. Death of dopaminergic neurons in the substantia nigra, for example, is associated with the symptoms of Parkinson's disease. In the last ten years the number of putative neurotransmitters has increased by a factor of four or five. Most of the newly detected chemical messengers are peptides. Our knowledge of the anatomy, physiology and pharmacology of peptidergic neurons is comparatively incomplete at present; indeed, it is clear that many biologically active peptides remain to be isolated and characterized. The work outlined above is principally devoted to improving our understanding of cells. To the extent that we understand these cells, we can formulate better hypotheses about their role in causing disease.

Proposed Course

The work outlined above is still in progress and will be continued.

Publications

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- Moskal, J. and Schaffner, A.E.: Monoclonal antibodies to the dentate gyrus: immunocytochemical characterization and flow cytometric analysis of hippocampal neurons bearing a unique cell-surface antigen. J. Neurosci. 6: 2045-2053, 1986.
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- Young, W.S. III: Periventricular hypothalamic cells in the rat brain contain insulin mRNA. Neuropeptides 8: 93-97, 1986.
- Bonner, T.I., Buckley, N.J., Young, A.C., and Brann, M.R.: Identification of a family of muscarinic acetylcholine receptor genes. Science 237: 527-532, 1987.

- Brann, M.R. and Cohen, L.V.: Diurnal expression of transducin mRNA and translocation of transducin in rods of rat retina. Science 235: 585-587, 1987.
- Kiss, A., Palkovits, M., Antoni, F.A., Eskay, R.L., and Skirboll, L.R.: Neurotensin in the rat median eminence: the possible sources of neurotensin-like fibers and varicosities in the external layer. Brain Res. 416: 129-135, 1987.
- Kligman, D. and Hsieh, L.-S.: Neurite extension factor induces rapid morphological differentiation of mouse neuroblastoma cells in defined medium. Dev. Brain Res. 33: 296-300, 1987.
- Koller, K.J. and Brownstein, M.J.: Use of a cDNA clone to identify a supposed precursor protein containing valosin. Nature: 325: 542-544, 1987.
- Palkovits, M. and Eskay, R.L.: Distribution and possible origin of β -endorphin and ACTH in discrete brainstem nuclei of rats. Neuropeptides 9: 123-137, 1987.
- Palkovits, M., Leranath, C., Gorcs, T., and Young, W.S. III: Corticotropin-releasing factor in the olivocerebellar tract of rats: Demonstration by light- and electron-microscopic immunohistochemistry and *in situ* hybridization histochemistry. Proc. Natl. Acad. Sci. USA 84: 3911-3915, 1987.
- Rokaeus A.: Galanin: a newly isolated biologically active neuropeptide. Trends Neurosci. 10: 158-164, 1987.
- Young, W.S. III, Mezey, E., and Siegel, R.E.: Vasopressin and oxytocin mRNAs in adrenalectomized and Brattleboro rats: analysis by quantitative *in situ* hybridization histochemistry. Mol. Brain Res. 1: 231-241, 1986.
- Young, W.S. III, Walker, L.C., Powers, R.E., De Souza, E.B., and Price, D.L.: Corticotropin-releasing factor mRNA is expressed in the inferior olives of rodents and primates. Mol. Brain Res. 1: 189-192, 1986.
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- Young, W.S. III, Mezey, E., and Siegel, R.E.: Quantitative *in situ* hybridization histochemistry reveals increased levels of corticotropin-releasing factor mRNA after adrenalectomy in rats. Neurosci. Lett. 70: 198-203, 1986.
- Young, W.S. III, Bonner, T.I., and Brann, M.R.: Mesencephalic dopamine neurons regulate the expression of neuropeptide mRNAs in the rat forebrain. Proc. Natl. Acad. Sci. USA 83: 9827-9831, 1986.

Koller, K.J., Wolff, R.S., Warden, M.K., and Zoeller, R.T.: Thyroid hormones regulate levels of thyrotropin-releasing hormone mRNA in the paraventricular nucleus. Proc. Natl. Acad. Sci. USA 84 (in press), 1987.

Young, W.S. III, Warden, M.K., and Mezey, E.: Tyrosine hydroxylase mRNA is increased by hyperosmotic stimuli in the paraventricular and supraoptic nuclei. Neuroendocrinology (in press), 1987.

Lightman, S.L. and Young, W.S. III.: Changes in hypothalamic preproenkephalin mRNA following stress and opiate withdrawal. Nature (in press), 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02302-02 LCB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Studies on Myelin Basic Protein

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

R. E. Martenson

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cell Biology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been discontinued because the principal investigator left the Laboratory of Cell Biology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00422-16 LCB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropharmacology of Circadian Rhythms

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Zatz Section Chief SBP,LCB,NIMH

Others: N. Harrison Visiting Fellow LNP,NINCDS
 M. Warden Biologist LCB,NIMH

COOPERATING UNITS (if any)

LNP, NINCDS

LAB/BRANCH

Laboratory of Cell Biology

SECTION

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NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.9

PROFESSIONAL:

1.0

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Circadian rhythms and environmental lighting regulate a number of endocrine and behavioral functions. Dispersed chick pineal cells remain rhythmic and responsive to light in culture. Photosensitivity appears to reside in the same cells as melatonin production. The mechanisms of phototransduction appear to differ from those in retinal rod cells. Light, membrane potential, norepinephrine, cyclic AMP, and calcium channels regulate melatonin rhythms in these cells.

Project Description

Objectives: To elucidate the biochemical mechanisms and neuropharmacology of circadian rhythms; to elucidate the mechanisms by which light suppresses and entrains melatonin rhythms.

Methods: Biochemical, pharmacologic, electrophysiologic, histologic, cell culture, and radioactive trace techniques.

Major Findings: Several laboratories have demonstrated the persistence of photosensitive rhythms related to melatonin secretion in cultured chick pineals. We have developed a system using dispersed chick pineal cells in static culture, which displays a rhythm of melatonin release for at least two weeks under cyclic lighting conditions, and for at least 4 cycles under constant red light. Using a rapid and specific extraction assay for the ^{14}C -melatonin formed (from ^{14}C -tryptophan) and secreted by these cells, we have examined the effects of perturbations on the amplitude, period, and phase of the melatonin rhythm. With this approach, simultaneous comparisons of the effects of multiple, independent perturbations on virtually identical, cycling, photosensitive cells can be made.

The period in constant red light was close to 20 hours, but in constant white light (or 12:12 cycles) it was closer to 24 hours. Four hour pulses of white light (in otherwise constant red light) caused an acute fall in melatonin output, and phase-dependent phase shifts of the rhythm relative to controls. Pulses of darkness (in otherwise constant red light) tended to increase melatonin output, and caused phase-dependent phase shifts. Elevated potassium concentrations increased melatonin output and the amplitude of the rhythm, but did not change the period. Four hour pulses of low (5.4 mM) potassium (in otherwise constant high potassium) mimicked the acute effect of light, reducing melatonin output, but did not induce appreciable phase shifts. Changes in membrane potential appear more likely to be involved in the regulation of melatonin output (and thus be regulated by the pacemaker) than to be involved in regulation of the pacemaker which generates the melatonin rhythm.

The acute suppressive effects and phase-shifting effects of light indicate a pathway from the photopigment to the pacemaker generating the melatonin rhythm. One mechanistic model for such a pathway derives from the retinal rod photoreceptor. There, regulation of cyclic GMP levels is a critical step in phototransduction. In the chick pineal, addition of 8 Br-cyclic GMP had minimal effects on the level, period, or phase of melatonin production. In contrast, 8 Br-cyclic AMP markedly increased melatonin production, without changing its phase or period. These results suggest that light acts on the chick pineal by two mechanisms, an acute suppressive effect involving cyclic AMP and an effect on the pacemaker not clearly mediated by the cyclic nucleotides. The mechanism of phototransduction in retinal rods does not appear to be used for either effect. Furthermore,

norepinephrine, acting through an α_2 -adrenoceptor, mimicked the acute suppressive effect of light but did not cause phase shifts in the rhythm of melatonin release. In view of other evidence suggesting that norepinephrine's effect is mediated by the GTP-binding protein which inhibits adenylate cyclase (G_i), these results support the inference that cyclic AMP is involved in the regulation of melatonin production by the pacemaker but not in the regulation of the pacemaker by light.

The nocturnal increase in melatonin output was also suppressed both by inorganic calcium channel blockers (Co or Mn) and by dihydropyridine antagonists; conversely, melatonin output was enhanced by the calcium channel 'agonist' BAY K 8644. These data suggest the existence of 'L-type' Ca channels in the plasma membrane of these cells. Electrophysiological recordings were made from individual chick pineal cells after 2-3 days in culture, using the whole-cell patch-clamp technique. The cells are of high input resistance (1 Gohm), and do not generate TTX-sensitive action potentials. No transient outward current was observed, but all cells exhibit a TEA-sensitive sustained outward current. In the presence of TEA, presumed Ca-dependent action potentials are evoked by depolarizing current injection. When Ca currents were isolated, they were found to consist of two components, one sustained and the other rapidly inactivating. Both components of inward current declined at test potentials positive to +10mV and were abolished by 2mM Co. When the holding potential was -40mV, only a sustained inward current was activated during depolarizing commands. This was enhanced by BAY K 8644 and blocked by nifedipine. The inactivating current was insensitive to dihydropyridines. These two Ca currents are similar to those previously designated L-type (sustained) and N-type (transient). The above results indicate that voltage-dependent Ca channels are important in controlling the entry of Ca into, and the regulation of melatonin output from, chick pineal cells.

Another question we addressed this year was whether the photoreceptive mechanism resides in the same cells as melatonin production. Immunocytochemistry revealed the presence of membrane-bound opsin and alpha-transducin immunoreactivity in virtually all the non-fibroblast chick pineal cells in culture. These results suggest that the majority of pinealocytes in the dish are photosensitive. Immunostaining with an antibody directed against HIOMT, the final enzyme in melatonin biosynthesis, also showed immunoreactivity in virtually all the non-fibroblast cells. Taken together, these results strongly suggest that regulation of melatonin synthesis by light does not require intercellular communication but is a cellular property of chick pinealocytes.

Significance to Biomedical Research: Circadian rhythms occur in hormone levels, activity, mood, etc. and are primarily regulated by light-dark cycles. The mechanisms generating and regulating circadian rhythms are of broad clinical and biologic interest. This photosensitive cultured cell system, with its biochemically measurable output, has unique advantages for the investigation of

biochemical mechanisms regulating phototransduction and circadian rhythmicity.

Proposed Course of Project: The relationship between cyclic AMP and calcium influx in the regulation of melatonin biosynthesis, will be explored. Whether the effects of light and norepinephrine are mediated by GTP-binding proteins will be determined. Pharmacological agents acting on the pacemaker will be sought; agents acting on calcium and other ion channels, ion-exchange mechanisms, phospholipases, and protein synthesis will be tested for direct effects on the pacemaker and for interactions with the effects of light. If feasible, mechanisms regulating melatonin in chick and rat pineal will be compared. If feasible, dynamic regulation of the photopigment and transducin will be sought at the protein and mRNA levels.

Publications:

Zatz, M., Mullen, D.A., and Moskal, J.R.: Photoendocrine transduction in cultured chick pineal cells: Effects of light, dark, and potassium on the melatonin rhythm. Brain Res., (in press).

Zatz, M.: Pondering the pineal in chick vs. rat. In Sandler, M. (Ed.): Proceedings of the 6th International Catecholamine Symposium. New York, Alan R. Liss, (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00429-08 LCB

PERIOD COVERED

October 1, 1986 - September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemistry of Membranes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Zatz Section Chief SBP, LCB, NIMH

Others: L.C. Mahan Staff Fellow LCB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cell Biology

SECTION

Section on Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.2

PROFESSIONAL:

0.1

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

1.) Previous work explored the mechanisms by which lithium causes ACTH secretion from anterior pituitary tumor cells. Departure of one of the investigators and technical difficulties have delayed further work on this problem.

2.) Previous work demonstrated the acylation of rhodopsin by long chain fatty acids in vivo and in vitro. Further work on this problem waits identification of the opsin mediating photoreception in cultured chick pineal cells which will provide a model system for the exploration of the role of this new class of posttranslational modification in receptor function.

Project Description:

Objectives: To determine the mechanisms by which lithium stimulates and desensitizes ACTH secretion from cultured anterior pituitary cells. 2) To elucidate the nature and function of protein acylation.

Methods Biochemical, chromatographic, enzymatic, pharmacologic, cell culture, and radioactive trace techniques.

Major Findings: None this year.

Significance to Biomedical Research: 1) A stimulatory effect of lithium, at therapeutic concentrations, is unusual. Elucidation of the mechanism of action of lithium on ACTH secretion could shed light on the therapeutic actions of lithium as well as on the regulation of ACTH secretion. 2) Acylation of membrane proteins provides a mechanism for posttranslational modification of the lipophilicity of receptors, ion channels, etc. which could alter their function and regulate their interactions with cell membranes, other proteins, or signal molecules.

Proposed Course of Project:

1) Lithium's interactions with calcium and ion channels will be investigated. 2) The role of acylation in regulation of membrane receptor turnover and function will be explored.

Publications:

Zatz, M. Translocation of protein kinase C in rat hippocampal slices. Brain Res. 385: 174-178, 1986.

Zatz, M., Mahan, L.C., and Reisine, T.: Translocation of protein kinase C in anterior pituitary tumor cells. J. Neurochem. 48, 106-110, 1987.

O'Brien, P.J., St. Jules, R.S., Reddy, T.S., Bazan, N.G. and Zatz, M.: Acylation of disc membrane rhodopsin may be non-enzymatic. J. Biol. Chem. 262: 5210-5215, 1987.

Zatz, M. and Reisine, T.: Corticotropin (ACTH) secretion. In Lithium therapy Monographs, Vol. II, Lithium and the Endocrine System, F.N. Johnson, ed., Karger Medical Publishers, N.Y., (in press).

Reisine, T. and Zatz, M.: Interactions between lithium, calcium, diacylglycerides and phorbol esters in the regulation of ACTH release from AtT-20 cells. J. Neurochem., (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00427-10 LCB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

On the Mechanism of Signal Transduction Through Receptors

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

F. Hirata

COOPERATING UNITS (if any)

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Laboratory of Cell Biology

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INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been discontinued because the principal investigator left NIMH.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00434-06 LCB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms of Receptor-Mediated Signal Transduction

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Julius Axelrod	Guest Researcher	LCB, NIMH
Others:	R. Burch	Guest Researcher	LCB, NIMH
	B. Conklin	Guest Researcher	LCB, NIMH
	C. Felder	Staff Fellow	LCB, NIMH
	C. L. Jelsema	Guest Researcher	LCB, NIMH
	A. D. Ma	Guest Researcher	LCB, NIMH
	A. L. Ma	Biologist	LCB, NIMH
	L. Mahan	Staff Fellow	LCB, NIMH

COOPERATING UNITS (if any)

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Laboratory of Cell Biology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

5.0

PROFESSIONAL:

5.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

GTP-regulatory proteins, in particular transducin, mediate light-induced stimulation of phospholipase A₂ and C (PLA₂, PLC) in rod outer segments (ROS) of bovine retina. Studies using G protein-specific agents, cholera toxin and pertussis toxin, in both light and dark-adapted ROS suggest a dual role for G proteins in both activation and inhibition of PLA₂ and PLC. $\beta\gamma$ subunits of transducin stimulated PLA₂ while α subunits were inhibitory. Both α and $\beta\gamma$ subunits of transducin stimulated PLC, possibly through the inhibition of a inhibitory G protein. In addition to light, stimulation of PLA₂ and PLC by other retinal neurotransmitters, in particular dopamine and somatostatin has been characterized. Isolated α subunits from G_s, G_i, G_o and transducin were phosphorylated *in vitro* by cAMP-dependent protein kinase and protein kinase C. Phosphorylated subunits were altered in their ability to stimulate phospholipase activity and this may represent a biochemical mechanism of regulation of G protein-mediated pathways. In retina from *Xenopus laevis*, both somatostatin and dopamine inhibit the circadian rise in N-acetyl transferase (NAT), the rate limiting enzyme in melatonin synthesis. These effects are mediated by G proteins although through different transduction mechanisms. Somatostatin, acts through a non-cAMP dependent mechanism. Receptors for somatostatin are regulated by Na⁺ and GTP and co-localize with G proteins, in particular G_o, by autoradiographic and immunocytochemical analyses respectively. In addition, the ability to immunocytochemically detect G protein subunits is markedly altered during peak periods of circadian activity.

Activation of PLA₂ and PLC by bradykinin has been shown to occur through distinct G proteins in Swiss 3T3 cells. Activation of PLA₂ occurs via a pertussis toxin-insensitive G protein. Evidence to support the existence of two distinct bradykinin receptor subtypes coupled to the activation of phospholipases was obtained.

Project Description:Studies of phospholipase A₂ and phospholipase C in receptor-mediated signal transduction.

Dr. Jelsema has demonstrated that in the rod outer segments of bovine retina, both phospholipase A₂ and phospholipase C are under regulation by GTP-binding proteins, including the retinal G protein, transducin. The $\beta\gamma$ subunits of transducin function in activation of phospholipase A₂ while the α subunit inhibits this effect. The mechanism for phospholipase A₂ stimulation by the $\beta\gamma$ subunits is currently under investigation. In the modulation of phospholipase C, both the transducin α and $\beta\gamma$ subunits are stimulatory, and involve the inactivation of an inhibitory G protein. Dr. Jelsema is currently attempting to identify this inhibitory G protein and characterize the mechanism whereby the transducin α and $\beta\gamma$ subunits activate phospholipase C. Since the $\beta\gamma$ subunits are common to all G proteins, the activation of phospholipase A₂ and C by these subunits has implications for all signal-transducing systems that employ G proteins.

Dr. Jelsema, in collaboration with A.D. Ma, have demonstrated the existence of three distinct phospholipase C enzymes in rod outer segments of bovine retina, each of which appears to be regulated by different G proteins as evidenced by the different effects of light, GTP γ S and pertrussis and cholera toxin on the hydrolysis of phosphatidylinositol, phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate. In collaboration with Dr. S. Rhee (NHLBI), she is currently attempting to identify these phospholipases using specific antibodies. These studies, while revealing unexpected complexities, are important to understanding the regulation of this second messenger system.

Dr. Jelsema, in collaboration with A.D. Ma, has examined the role of G protein subunits other than transducin in the modulation of phospholipase A₂ activity using a reconstituted system that consists of isolated, purified G protein subunits, porcine pancreas phospholipase A₂ and phosphatidylcholine vesicles. Future studies in collaboration with Dr. C. Felder are designed to isolate the membrane-bound phospholipase A₂ of the rod outer segments for reconstitution with the G protein subunits, as described above. It is only studies of this nature will enable the identification of the G proteins responsible for regulation of these phospholipases.

Dr. Jelsema, in collaboration with A.D. Ma and L. Mahan, have investigated the role of retinal neurotransmitters, dopamine and somatostatin, respectively in modulation of phospholipase A₂ and C. Dopamine has recently been identified as a major neurotransmitter in the retina and these studies may provide a clue as to the action mechanism. G protein-dependent regulation of these enzymes by D2 dopamine receptors is independent of its

inhibitory effect on adenylate cyclase, the classical mechanism of action of D2 receptors. Preliminary observations indicate that somatostatin also stimulates phospholipase A₂ and C in these membranes.

Dr. Jelsema, in collaboration with Dr. D. Rausch, have discovered that nerve growth factor (NGF) inhibits phospholipase A₂ while stimulating phospholipase C activity in PC12 pheochromocytoma cells. They are currently examining the effect that transfection with ras or src has on these two phospholipases since these transfections, similar to NGF treatment, lead to neurite extension and both ras expression as well as src-induced kinase activity have been implicated in the regulation of these two phospholipases. These studies should provide clues not only to the role of phospholipases in the mode of action of these oncogenes but also the role of phospholipases in neuronal development and the mechanism of action of NGF.

Dr. Jelsema, in collaboration with Dr. D. Lewis (NINCDS), is examining the effect of GTP-binding protein subunits on the voltage-sensitive, G protein-regulated Ca⁺⁺ channels present in AtT20 mouse pituitary cells. They are also examining the role of phospholipase A₂ and the role of specific arachidonate metabolites in the somatostatin-mediated opening of the Ca⁺⁺ channels. These studies are aimed at examining the mechanism whereby G proteins regulate ion channels.

Studies of protein kinase C modulation of GTP-binding proteins:

Dr. Jelsema, in collaboration with Drs. R. Kahn (NCI), S. Jakens (Alton Jones Cell Science Center, Lake Placid, New York) and A.D. Ma have analyzed the in vitro phosphorylation of the GTP-binding proteins G_s, G_i, G_o and transducin using 3 distinct isozymes of protein kinase C and cAMP-dependent protein kinase. Only the G protein α subunits were found to be substrates for the kinases, with the kinases exhibiting marked differences in substrate specificity. The functional effect of the G protein phosphorylation was examined in terms of the capacity of the G proteins to modulate phospholipase A₂ activity in a reconstituted system. These studies illustrate the possibility that desensitization of a receptor-mediated signal may also occur at the level of the G protein.

Neurotransmitter Regulation of circadian rhythm in retina

Dr. Mahan has demonstrated the presence of somatostatin (SRIF) receptors in retina from *Xenopus laevis* and shown that SRIF can inhibit the circadian rhythm of N-acetyltransferase activity and melatonin synthesis in in vitro eye cup preparations. These receptors are coupled to a G protein that mediates inhibition in a non-cAMP dependent fashion unlike the inhibition by dopamine via D2 receptors in this tissue. Current experiments are attempting to define this inhibitory transduction

system and the role of SRIF in the regulation of the circadian clock. In collaboration with Drs. Jelsema and Palkovits, studies are being performed to identify the nature and distribution of the G proteins in these retina during the circadian cycle.

In collaboration with Drs. Brownstein, Okayama, Buckley, Lolait and Koller, Dr. Mahan has been screening mammalian cells (mouse L(A9)) transfected with sized and unsized cDNA libraries from anterior pituitary, AtT-20 and AR42J cells which are rich in somatostatin receptors. This method utilizes a cell colony replication/ligand-binding assay capable of screening > 100,000 colonies in an assay. Dr. Mahan hopes to address whether the multi-transductional nature of SRIF receptors and their distribution in both peripheral and CNS tissues are the consequence of a single gene product.

In collaboration with Drs. Koller and Bonner, Dr. Mahan has begun to screen sub-libraries from the above mentioned cDNA libraries by hybridization (southern) analysis using a 56-oligonucleotide probe developed in this laboratory. This probe has sequence homology to muscarinic and beta-adrenergic receptors. These receptors as well are linked to G-protein-mediated transduction systems and may make the use of this "consensus" probe a valuable approach for other G protein-linked receptors.

In collaboration with Dr. Zatz, Dr. Mahan has conducted experiments on the mechanism of Li⁺-stimulated secretion of ACTH from AtT-20 cells.

Bradykinin stimulated phospholipase activation and control of prostaglandin synthesis in swiss 3T3 cells and CPAE endothelial cells.

Dr. Ronald Burch has shown a dissociation of bradykinin-induced activation of phospholipase A₂ (leading to prostaglandin formation) and phospholipase C (leading to phosphatidylinositol turnover) in Swiss 3T3 cells. Further evidence was obtained using GTP analogs which indicated that a pertussis toxin insensitive G protein regulates phospholipase A₂ activation by bradykinin.

Bruce Conklin has characterized the differential effects of several bradykinin analogs on the activation of phospholipases A₂ and C in Swiss 3T3 cells and CPAE cells. The effects of these bradykinin analogs on prostaglandin synthesis do not fit the previously described B₁, B₂ bradykinin receptor classification. These findings indicate that there are at least two bradykinin receptors which stimulate prostaglandin synthesis, one present in Swiss 3T3 cells coupled to phospholipase A₂, and the other in CPAE cells coupled to phospholipase C.

Significance to Biomedical Research: A clear understanding of the action of psychoactive agents that mimic or interfere with

receptor-mediated functions in nervous tissue requires elucidation of post-receptor mechanisms of signal transduction. Guanine nucleotide-binding proteins modulate a variety of cellular responses to both hormones and neurotransmitters. The role of G proteins in receptor-mediated stimulation and inhibition of cAMP-dependent processes is best described. More recently, these proteins have been shown to be involved in cAMP-independent processes such as ionic conductance, Ca^{++} mobilization and phospholipid metabolism. These additional mechanisms of signal transduction may act apart from or in concert with cAMP to effect such diverse biological responses as hormone secretion, circadian rhythm and cell growth. Alterations in or loss of cellular responsiveness (e.g., desensitization), once associated only with changes in receptor number, can also reflect the state of coupling between receptor and the G protein that mediates its biological response.

Proposed Course of Project:

1. Future studies on AtT-20 cells will focus on isolation and characterization of SRIF receptors and the nature of the G proteins that couple these receptors to cAMP-dependent and -independent pathways of inhibition of ACTH secretion. Receptor isolation by expression/cloning from selected cDNA libraries in eukaryotic vectors will continue. In addition screening of these libraries with "consensus" G protein-linked receptor probes (30-56 bp) has begun. Development of unique SRIF derivatives to enable studies of receptor expression and regulation in the intact cell will continue.

2. Future studies in retina will focus on the mechanism of action for transducin-mediated increases in phospholipases A_2 and C. In particular, Dr. Jelsema will examine the effects of various protein kinases on the phospholipase-stimulating activities associated with the transducin. Collaborations with Drs. J. Axelrod, E. Jelsema and L. Mahan are continuing to describe the interaction of various neurotransmitters with the phospholipases of the ROS of bovine and frog retina. Activation of D2 dopamine and somatostatin receptors mimic the stimulatory effect of light in dark-adapted ROS. Using antibodies specific for transducin and G protein subunits, Dr. Jelsema will focus on whether the activation of the phospholipases by these neurotransmitters similarly involves transducin or is coupled to other G proteins. These antibodies are also being employed to identify the G proteins involved in the inhibition of phospholipases A_2 and C. Analysis of the modulating effect of various protein kinases on G protein functions is being continued with Dr. S. Jakens of the FDA.

Alternative inhibitory mechanisms of SRIF (e.g., Ca^{++} mobilization, arachidonic acid release, phosphoinositide metabolism) in frog retina are currently being explored. In addition, the role of G proteins in modulating the endogenous circadian rhythm in these retina will be investigated.

Future studies in Swiss 3T3 cells and CPAE endothelial cells will focus on the mechanism by which phorbol esters activate phospholipase A₂. In addition investigations have been initiated to study the effects of bradykinin on arachidonic acid metabolism in primary culture rat astrocytes.

Publications:

Burch, R.M. and Axelrod, J.: Disassociation of bradykinin-induced prostaglandin formation from phosphatidylinositol turnover in Swiss 3T3 cell: evidence for G protein regulation of phospholipase A₂. Proc. Natl. Acad. Sci. USA, in press, 1987.

Mahan, L.C. and Reisine, T.D.: Molecular Mechanisms of Somatostatin Inhibition of Hormone Release from AtT-20 Cells. In Reichlin, S. (Ed.): Somatostatin: Basic and Clinical Status. New York, Plenum Press, 1987, pp. 137-145.

Mahan, L.C., McKernan, R.M. and Insel, P.A.: Metabolism of alpha- and beta-adrenergic receptors in vitro and in vivo. Ann. Rev. Pharmacol. Toxicol. 27: 215-235, 1987.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02385-01 LCB

PERIOD COVERED

October 1, 1986 - September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Control of Cell Differentiation, Growth and Transformation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: H. Okayama Visiting Scientist LCB, NIMH

Others: M. Kawaichi Visiting Associate LCB, NIMH
M. Eiden Guest Researcher LCB, NIMH
A. Masuda Visiting Fellow LCB, NIMH
C. Chen Biologist LCB, NIMH
N. Nukiwa Guest Researcher LCB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cell Biology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.4

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A highly efficient transfection protocol and a plasmid vector for the construction and delivery into mammalian cells of cDNA expression libraries have been developed. Many commonly used fibroblastic cell lines are stably transfected at frequencies of >10% with the cDNA cloning vector that incorporates a neo selectable marker. The system permits cloning of cDNAs on the basis of their function expressed in mammalian cells.

The majority of human cancers seem to be induced by a recessive mechanism (recessive oncogenes). To clone such a novel type oncogene, nitroso methylurea-transformed BHK cells were transfected with a cDNA expression library constructed with the new vector and mRNA prepared from primary human fibroblast cells. After neo selection and morphological screening, two flat revertants that were unable to grow in soft agar were isolated. In secondary transfection with the genomic DNA prepared from one of the two revertants, the flat phenotype co-transmitted with neo resistance. The cDNA that induces flat reversion is being recovered in E. coli for molecular cloning and characterization.

Project Description:

Objectives: To elucidate the molecular mechanism of cell differentiation, growth control and malignant transformation: cloning of cellular oncogenes and genetic elements involved in transformation, and genes for growth and differentiation factor signal transduction pathways.

Methods employed: Recombinant DNA, molecular cloning, cell culture, and gene analysis techniques.

Major Findings:

1) Development of a high-efficiency transfection method and a cDNA cloning expression vector with a selectable marker gene.

It is extremely important to develop a general method that permits cloning of genes on the basis of their functions expressed in appropriate host cells. Cellular genes that can be detected with conventional probes (oligonucleotides synthesized on the basis of the primary sequence of the protein product, or antibody) are increasingly rare. Virtually no such probes are available for genes that regulate cell growth and differentiation. For this reason, we developed a method for cloning full-length cDNA in an expression vector. This year we have devised a calcium phosphate-mediated DNA transfection method that achieves transformation frequencies of double-digit numbers, and constructed pcD2, a neo marker-containing cDNA cloning expression vector, which takes full advantage of this method. This transfection-vector system is almost as efficient as retrovirus vectors, yet more versatile, and is suitable for delivering cDNA libraries into mammalian cells for expression cloning of cDNA.

2) Molecular cloning of Recessive Oncogenes.

There is ample evidence that the majority of human cancer is caused by a recessive mechanism (recessive oncogenes) unlike virus-caused cancer, which is mediated by dominant oncogenes or transforming genes incorporated in the viruses. One of the best defined in vitro system to study transformation by recessive mechanism is nitroso methylurea (NMU) transformed BHK cells developed by Noel Bouk. et al. Treatment with nitroso methylurea, a chemical mutagen, induces transformation of BHK cells by inactivating a single gene. The resulting transformed cells are phenotypically recessive. Upon fusion with normal BHK or human fibroblast cells, the transformed cell property is suppressed through genetic complementation. We have been using this cell system to clone a recessive oncogene. A cDNA expression library was constructed with the newly developed pcD2 vector and mRNA from primary human fibroblasts. The library was transduced into NMU-transformed BHK, and stably transfected cells were selected in the presence of G418. The transfected cells were screened for morphologically flat cells under a

microscope. Two flat revertants were isolated and both were found to be unable to grow in soft agar. Secondary transfection of the original transformed BHK with the total genomic DNA prepared from one of the flat revertants yielded neo-resistant colonies with similar flat phenotypes (unable to grow in soft agar), indicating that the gene that induced the flat phenotype is physically linked to the neo gene present in the vector and therefore, very likely to be the integrated cDNA. Recovery in E. coli, expression assay, and structural analysis of the cDNA are currently in progress.

3) Transformation of C3H10T1/2 cells by an epigenetic mechanism.

Whether cells can be transformed by epigenetic mechanisms has been one of the central questions in cancer research. During the course of studies of chemically transformed C3H cells, we have found that co-culturing normal C3H cells with methylcholanthrene-transformed C3H(MB66) leads to morphological transformation of the C3H cells. The transformation is partially reversible and seems to be mediated by either a diffusible factor(s) or factor(s) transmitted by direct cell-cell contact. Conditioned medium obtained from the culture of the transformed C3H is almost inactive. MB66 cells are reverse transcriptase-negative, excluding the possibility that the factor is a transforming retrovirus. We are currently identifying the factor.

4) The transforming growth factor-beta (TGF-beta) signal transduction pathways.

TGF-beta has been suggested to play a major role in the control of cell growth, terminal differentiation and transformation. TGF-beta has extremely diverse actions: induction or suppression of differentiation, growth suppression, and transformation, depending on what cells are used for assay. At least three distinct receptors for TGF-beta have been identified on cell membrane. In order to understand the TGF-beta signal transduction pathways that induce such diverse biological actions, we have begun to isolate TGF-beta insensitive cell mutants for genetic studies of the pathways and for use as hosts for complementation assay to clone genes that constitute the pathways.

Significance to Biomedical Research: The development of the gene cloning and transfection techniques should greatly facilitate isolation and characterization of genes involved in various fundamental cell functions. Studies of genes involved in oncogenesis, cell growth and differentiation are essential to the understanding of the mechanism of these cell functions and, ultimately, the pathogenesis of various diseases caused by disorders of these functions.

Proposed Course of Project: The possibility to use yeast as

an expression host to screen cDNA libraries will be explored. The cDNA will be recovered from the flat revertant of NMU-transformed BHK, and its gene and protein structure, its tissue- or development-specific expression and the function of the coded protein will be examined. The factor that induces transformation of C3H cells will be identified.

Publications:

Okayama, H., Development and application of a vector system that permits cloning of cDNAs based on their functional expression in mammalian cells. In Umeda, M., Koyama, H., Oishi, M., and Minowada J. (Eds.): Biotechnology of Animal Cells. Tokyo, Japan Scientific Societies Press, pp. 91-101, 1987.

Okayama, H.: Phage-mediated transduction of cDNA libraries into mammalian cells. Methods Enzymol. 151: 434-444, 1987.

Okayama, H., Kawaichi, M., Brownstein, M., Lee, F., Yokota, T., and Arai, K.: High-efficiency cloning of full length cDNA; Construction and screening of cDNA expression libraries for mammalian cells. Methods Enzymol. 154: 3-28, 1987.

Chen, C., and Okayama, H.: High-efficiency transformation of mammalian cells by plasmid DNA. Mol. Cell. Biol. (in press), 1987.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02386-01 LCB

PERIOD COVERED

October 1, 1986 through September 30, 1987.

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropeptide Secretion, Synthesis and Action in Neural, Endocrine and Immune Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Lee E. Eiden Pharmacologist LCB, NIMH

See Attached

COOPERATING UNITS (if any)

U. Strasbourg; D.C. V.A.; LDN, NICHHD; NIAAA; U. Innsbruck, UCSF; A. Einstein Coll. Med.; Merrell-Dow Res. Inst.; Case Western Reserve; USUHS

LAB/BRANCH

Laboratory of Cell Biology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

6

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are examining the molecular mechanisms of neuropeptide secretion, neuropeptide expression and biosynthesis and neuropeptide interactions with their receptors in the developing and mature neuroendocrine system. We are attempting to understand the structural features of peptides and proteins that confer molecular specificity on these three processes. We hope to characterize and develop pharmacological agents that mimick this specificity.

Other Professional Personnel Engaged on Project:

D. Aunis	Directeur de Recherche	U. Strasbourg
M.F. Bader	Charge de Recherche	U. Strasbourg
D. Breneman	Pharmacologist	LDN,NICHH
M. Brownstein	Chief	LCB,NIMH
J. Dave	Visiting Scientist	NIAAA
J. Disbrow	Guest Researcher	LCB, NIMH
R. Eskay	Chief, Sect. on Neurochem	NIAAA
R. Fischer-Colbrie	University Asst.	U. Innsbruck
M. Grimes	Fellow	UCSF
C.M. Hsu	Biologist	LCB,NIMH
A. Iacangelo	Microbiologist	LCB,NIMH
J. Moskal	Assoc. Professor	A.Einstein Coll. Med.
J.M. Muller	Guest Researcher	LCB,NIMH
H. Okayama	Visiting Scientist	LCB,NIMH
D. Perrin	Guest Researcher	LCB,NIMH
R. Pruss	Senior Scientist	Merrell- Dow Res.Inst.
D. Rausch	Guest Researcher	LCB,NIMH
A. Rokaeus	Visiting Fellow	LCB,NIMH
R. Siegel	Asst. Professor	Dept. Pharmacology, Case Western Reserve
P. Smith	Asst. Professor	Dept. Anatomy, USUHS
K. Timmers	Assoc. Professor	Georgetown, D.C. V.A.
J. Waschek	Guest Researcher	LCB,NIMH

Project Description:

The Unit of Molecular and Cellular Neurobiology, as a part of the Laboratory of Cell Biology, investigates the molecular mechanisms regulating biosynthesis and secretion of neuropeptide hormones. Earlier, we have defined "stimulus-secretion-synthesis coupling" as the process by which secretagogues stimulate both secretion and synthesis of peptide hormones in neuroendocrine cells. Thus, cholinergic stimulation of chromaffin cells causes a concomitant release of enkephalin peptides and an increase in enkephalin synthesis as measured by an increase in enkephalin peptides within the cell and a rise in cellular enkephalin mRNA levels. Both these events are calcium-dependent, suggesting that calcium is the common second messenger subserving the coupling of enkephalin secretion and biosynthesis. In the past year, these findings have been extended in several ways. Drs. Eiden, Dave and Eskay have demonstrated that "stimulus-secretion-synthesis coupling" occurs in other endocrine cell types. Corticotrophs of the anterior pituitary employ cyclic AMP as the common second messenger coupling secretion and synthesis of pro-opiomelanocortin in response to corticotropin-releasing factor; calcium playing only a permissive role. Calcium is the common second messenger coupling secretion and synthesis of prolactin in pituitary lactotrophs. Waschek and his co-workers have demonstrated, using the calcium agonist barium, that distinct calcium targets within the cell separately mediate the stimulation of secretion and enhanced biosynthesis of enkephalin and VIP in chromaffin cells, and prolactin in pituitary

lactotrophs . Hsu and co-workers have demonstrated that both dihydropyridine-sensitive and insensitive channels operate in stimulus-secretion-synthesis coupling. Dr. Perrin, in collaboration with Dr. Smith, has developed an assay for observing secretion from individual chromaffin cells microscopically, and for injecting into single chromaffin cells agents which can perturb or mimic the events leading to calcium-dependent secretion. Dr. Perrin will attempt to determine the epitopes of the fodrin molecule important in neurosecretion by injection of fodrin peptide fragments into chromaffin cells, and whether or not calmodulin participates in secretion by injection of calmodulin antibodies and protein fragments.

Drs. Pruss, Eiden and Rokaeus have demonstrated that the mRNA for the neuropeptide galanin is abundantly expressed in bovine chromaffin cells and increased by phorbol esters (PMA). Enkephalin mRNA on the other hand, is regulated much less strongly, if at all, by PMA in the same cells that contain galanin. In addition, treatment with PMA causes a blockade of potassium-evoked stimulation of enkephalin mRNA . Drs. Eskay and Eiden, and Drs. Pruss and Zamir, have used the chromaffin cell in culture as a model to show that neurotensin, substance P, atrial natriuretic hormone, and NPY-like immunoreactivity are differentially affected by cyclic AMP, phorbol ester, and calcium. Drs. Wascheck and Pruss have also demonstrated that phorbol ester and forskolin act synergistically to increase endogenous VIP synthesis and exogenous VIP gene activation in neuroblastoma cells. Using primary cultures of mouse spinal cord as a model for the developing nervous system. Drs. Brenneman and Foster of the NIHCD and Dr. Eiden have collaborated to show that enkephalin and VIP, are biosynthesized at a high constitutive level which is dependent on the maintenance of spontaneous electrical activity (neuronal firing) in the cultures. We will try to learn from this model if neuronal activity determines the number of neurons expressing a given peptide in the developing spinal cord. Further experiments will be directed towards the role of calcium and specific neurotransmitter-coupled receptor activation in this process. Dr. Timmers has studied the regulation of enkephalin and insulin secretion and mRNA levels by second messengers and insulin secretagogues in rat insulinoma (RIN) cells. Drs. Timmers and Rokaeus have shown that insulin secretion is inhibited by galanin. Galanin levels are altered in the pancreas of obese rats in which insulin secretion is impaired. Direct inhibition of insulin secretion may be a part of the role of galanin in vivo. Enkephalin mRNA levels are stimulated synergistically by phorbol esters and cyclic AMP in RIN cells, whereas insulin mRNA appears to be expressed constitutively. These data suggest that neuropeptide expression and biosynthesis may be regulated in a developmentally and tissue-specific way by several receptor-linked second messenger systems. This hypothesis may be useful in understanding the ontogeny of neuropeptide diversity in the nervous system. Dr. Dianne Rausch has developed methodology which will greatly aid in studying the role of neurotransmitter and growth factor receptor

activation in terminal differentiation of neuroendocrine cells. She has constructed retroviral vectors expressing src and ras oncogenes, both of which cause high-efficiency infection and differentiation of murine pheochromocytoma cells. These vectors can now be used for genetic complementation of the differentiating function of cyclic AMP, nerve growth factor and epidermal growth factor. Based on previous reports that src overexpression in developing neurons may delay the onset of terminal differentiation, Drs. Rausch and Moskal have used a temperature-sensitive src retroviral vector to infect fetal CNS neurons in culture. Infected cells have been maintained for several passages in vitro. These vectors may be useful for preparing clonal populations of developing neurons, in which the signals that cause neuronal differentiation can be studied. Dr. Rausch has identified, by Northern blot analysis, a messenger RNA species specific to renal tissue which is homologous, but not identical, to src which is developmentally regulated in rat cerebral cortex. She is currently screening a cDNA library constructed by Dr. Brownstein from the rat cortex to isolate and characterize this developmentally regulated, src-homologous messenger RNA species and the protein it encodes.

Chromogranin A, whose primary sequence was determined last year by Ms. Iacangelo and her co-workers is a marker for developing chromaffin tissue in the autonomic nervous system, and a secretory protein whose biosynthesis, unlike the other neuropeptides described above, is not regulated by cyclic AMP, protein kinase C or calcium, but rather by glucocorticoids. Antibodies made against synthetic peptide fragments from the chromogranin A sequence appear to recognize a protein of similar size and isoelectric point in several metazoan and even a protozoan animal. Its evolutionary persistence and stability suggest that chromogranin A plays an important role in neuroendocrine function or secretory cell structure. The chromogranin protein structure, as deduced from the sequence of its cDNA, has now been determined for rat, and partially for human, as well as bovine chromogranin A (Iacangelo et al., in preparation; Grimes et al., in preparation). The hypothesis that chromogranin A is a prohormone for biologically active peptides is now being tested definitively by Dr. Perrin, by observing the effect of synthetic peptides, conserved in the sequence of chromogranin A from all three species, on the secretion of hormones from a variety of neuroendocrine cells in culture. Dr. Reiner Fischer-Colbrrie, on leave from the University of Innsbruck in Austria, has shown that chromogranin A expression in the adrenal gland is under the control of the pituitary, while enkephalin and neuropeptide Y expression are controlled by the splanchnic innervation of the adrenal gland (in preparation). The mRNA encoding chromogranin B, another acidic secretory protein of the adrenal gland is not altered by either splanchnic

firing or hypophysectomy. The adrenal medulla may contain at least three classes of neurosecretory proteins: those regulated by nerve traffic (enkephalin), those regulated by glucocorticoids (chromogranin A) and those constitutively expressed (neuropeptide Y). This work provides direct evidence for the concerted influence of hormonal and neural factors in determining the amount and type of each of several neuropeptides secreted by an endocrine organ. We are currently examining the hypothesis that humoral and neural factors together determine the basal secretory activity and peptide phenotype of several endocrine organs including the pancreas, pituitary gland and brain, using chromogranin A synthesis and secretion as a prototype. In addition, the gene encoding chromogranin A has been isolated and will soon be characterized, allowing an investigation of the sequences on the gene determining cell-specific expression and glucocorticoid regulation.

Jim Waschek has cloned and appended to a reporter gene the 5' regulatory sequences of the human VIP gene. He has discovered that a sequence between -2500 and -200 of the human VIP gene is responsible for its approximately 100-fold greater expression in human neuroblastoma compared to HeLa cells in vitro, and for its cell-specific transcriptional regulation by phorbol esters and cyclic AMP. (Waschek, J., Pruss, R.M., Hsu, C.M. and Eiden, L.E. *Neurosci.* 22 (Suppl): 5228, 1987. Drs. Waschek and Muller have also demonstrated in human neuroblastoma cells that VIP expression is inversely correlated to the expression of the VIP receptor. (Waschek, J. Muller, J.M., Hsu, C.M., Yu, V., Sudec, W. and Eiden, L.E. *Soc. Neurosci. Abstr.* 13: 1466, 1987, Muller, J.M., Eiden, L.E., Hsu, C.M. and Waschek, J.A. *Soc. Neurosci. Abstr.* 13: 1186, 1987). Since VIP receptor stimulation results in an elevation of intracellular cyclic AMP, which in turn stimulates IP gene expression, these data suggest an autocrine role for VIP in neuronal cells. Dr. Muller has also demonstrated that a CD4-positive human lymphoma cell line expresses high levels of the VIP receptor, and that this receptor is remarkably rapidly down-regulated by VIP at subnanomolar concentrations. Drs. Perrin and Muller are examining the role of cyto skeleton in mediating homologous and heterologous receptor down-regulation in these, and in neuroendocrine cells, in culture. The influence of VIP receptor down-regulation on the expression of other T-cell surface receptors, including CD4, is currently being examined. These cell lines should afford excellent model systems to test the hypothesis that VIP may regulate normal T-cell function and modulate T-cell-trophic viral infection of helper-inducer T-cells.

In collaboration with Dr. Tamir of Columbia University, the discoverer of SBP, Mr. Disbrow has begun the biochemical characterization of serotonin binding to SBP and fragments of the protein derived by cleavage with V8 protease. Preliminary experiments indicate that a serotonin-binding epitope of less than 20,000 daltons can be generated from the 45,000 dalton protein by protease treatment.

Significance to Biomedical Research:

Identification of the specific molecules that subserve neuropeptide secretion and synthesis, and the specific portions of each molecule involved in ligand-receptor interactions, will increase the likelihood of finding and designing specific pharmacological agents to mimic and to block these processes. These agents will allow further study of the structural features that impart specificity to the proteins involved in neuropeptide secretion and expression, the physiological consequences of blocking the secretion, synthesis or action of individual neuropeptides, and potentially specific blockade of pathogenic events mediated by proteins with similar types of structures (see below).

Proposed Course of Project:

The work described above will be followed to the endpoints of identifying pairs of molecules whose interaction within or on the cell is necessary for secretion, synthesis or action of specific neuropeptides, and designing and testing peptide fragments and analogues that mimic or block those interactions. To this end, we are currently developing methods for studying the behavior of individual neuropeptide secreting and synthesizing cells and the effects of injection of purified proteins, peptides and other factors into them.

Publications:

Brenneman, D.E. and Eiden, L.E.: Vasoactive intestinal polypeptide and electrical activity influence neuronal survival. Proc. Natl. Acad. Sci. USA 83: 1159-1162, 1986.

Dave, J.R., Eiden, L.E., Karanian, J. and Eskay, R.L.: Ethanol exposure decreases pituitary corticotropin-releasing factor binding, adenylate cyclase activity, proopiomelanocortin biosynthesis, and plasma B-endorphin levels in the rat. Endocrinology 118: 280-286, 1986.

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Ruth, J.A. and Eiden, L.E.: Enkephalins modulate chronotropic responses and calcium flux in rat and guinea pig atria. In: Stefano, G.B. (Ed.): Handbook of Comparative Aspects of Opioid and Related Neuropeptide Mechanisms v. 2. New York, CRC Press, 1986, pp. 91-102.

Dave, J.R., Eiden, L.E. and Eskay, R.L.: Differential effect of various secretagogues on B-endorphin release and pro-opiomelanocortin biosynthesis in rat anterior pituitary cells and AtT-20 cells. In: Puett, D., Ahman, F., Black, S., Lopez, D.M., Melner, M.H., Scott, W.A., Whelan, W.J. (Eds.): Advances in gene technology: Molecular biology of the endocrine system. Proceedings of the Eighteenth Miami Winter Symposium v. 4. Cambridge, ICSU Short Reports, 1986, pp. 34-35.

O'Donohue, T.L., Chronwall, B.M., Pruss, R.M., Mezey, E., Kiss, J.Z., Eiden, L.E., Massari, V.J., Tessel, R.E., Pickel, V.M., DiMaggio, D.A., Hotchkiss, A.J., Crowley, W.R. and Zukowska-Grojec, Z.: Neuropeptide Y and Peptide YY neuronal and endocrine systems. Peptides, in press, 1986.

Dave, J.R., Eiden, L.E., Lozovsky, D., Waschek, J.A. and Eskay, R.L.: Differential role of calcium in stimulus-secretion-synthesis coupling in lactotrophs and corticotrophs of rat anterior pituitary. Ann. N.Y. Acad. Sci. 493: 577-580, 1987.

Waschek, J.A., Pruss, R.M., Siegel, R.E., Eiden, L.E., Bader, M.F. and Aunis, D.: Regulation of enkephalin, VIP and chromogranin biosynthesis in actively secreting chromaffin cells: multiple strategies for multiple peptides. Ann. N.Y. Acad. Sci. 493: 308-323, 1987.

Grimes, M., Iacangelo, A., Eiden, L.E., Godfrey, B. and Herbert, E.: Chromogranin A: the primary structure deduced from cDNA clones reveals the presence of pairs of basic amino acids. Ann. N.Y. Acad. Sci. 493: 351-378, 1987.

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Dave, J.R., Eiden, L.E. and Eskay, R.L.: Elevation of intracellular cyclic AMP by corticotrophin-releasing factor links secretion of beta-endorphin and biosynthesis of pro-opiomelanocortin in cultured anterior pituitary and AtT-20 cells. Ann. N.Y. Acad. Sci., in press, 1987.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02387-01 LCB

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Analysis of the CD4/HIV Ligand/Receptor Dyad

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Lee E. Eiden Pharmacologist LCB, NIMH

Others: Jeff Lifson Director of Immunology Genelabs, Inc.
 Pat Padgett Chemist LMG, NINCDS
 Pete Nara Biologist FCRF, NCI
 Kou Hwang Chemist Genelabs, Inc.
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TOTAL MAN-YEARS:

1

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Peptide fragments of the CD4 antigen were synthesized and tested for anti-viral activity. A derivatized 19 amino acid fragment of the molecule inhibits HIV-mediated T-lymphocyte fusion and HTLV-IIIB infection of CEM cells, with an ED50 of 10-100 μ M in the presence of approximately 250TCID50 of the virus. The peptide is ineffective to block T-cell infection by HTLV-I, or to block other CD4-dependent cellular responses, e.g. the mixed lymphocyte reaction.

Project Description:

Z01 MH 02387-01 LCB

The CD4 antigen appears to be the receptor for the human immunodeficiency virus (HIV) in T-cell infection by this virus. We decided to investigate the structural requirements for this interaction because of our interest in peptide ligand-receptor interactions, and the presence of the CD4 antigen in both the immune system, where it subserves class-II-restricted T-cell helper function, and the central nervous system, where its function is unknown. In addition, the structures of both the gp120 ligand and the CD4 receptor are known, and functional assays for the ligand-receptor interaction exist, as do antibodies against both ligand and receptor. We have synthesized 20-25 amino acid fragments of the CD4 molecule, and tested these as competitive inhibitors of HIV infection and fusion of HIV-positive cells with uninfected CD4+ cells. None of the purified fragments were active to inhibit fusion of HTLV-IIIB-infected H9 lymphoma cells with VB CD4 positive indicator cells. A side fraction of the synthesis of one of the peptides did inhibit fusion completely at 125-250 μ M. Partial purification by differential extraction increased the nominal activity to 50 μ M. Chemical derivatization of the inactive parent peptide yielded preparations with nominal anti-viral activity of 60-120 μ M (complete blockade of fusion at these concentrations). The original material synthesized is also active in a direct assay for viral infection of CEM cells at a nominal concentration of 100 μ M (IC_{50} 10 μ M). We hypothesize that the active peptide is a side product of the original synthesis due to incomplete removal of a protecting group during HF cleavage of the peptide from the resin. The effect of the protecting group may be to force the remainder of the peptide to assume a conformation, which the free peptide does not, which is similar to the conformation of the peptide segment in the native protein.

Significance to Biomedical Research: The development of pan-specific anti-viral agents could result from further modifications of the core sequences found so far which are of modest potency but are fully efficacious to inhibit viral infection in vitro.

Proposed Course of Project: We intend to perform experiments designed to validate our original hypothesis, that a single continuous epitope of a protein receptor may act as an antireceptor agent if sterically constrained in the appropriate conformation. X-ray crystallographic and structure activity studies as well as anti-viral assays in vivo in STLV-infected macaque monkeys are planned.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02396-01 LCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanical, Thermal and Optical Signs Of Excitation In the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ichiji Tasaki Chief, Unit of Neurobiology LCB, NIMH

Others: Paul M. Byrne Biomedical Eng. Technician LCB, NIMH
 Michio Masumura Visiting Fellow LCB, NIMH
 (appointed Jan. 1987)

COOPERATING UNITS (if any)

LAB/BRANCH

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.5

PROFESSIONAL:

1.5

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We continued and expanded our investigation of non-electrical signs of excitation processes in the nervous system. Using isolated spinal cord preparations of the bullfrog and newborn rat, we found that afferent nerve impulses arriving at the spinal cord evoke a rapid rise in the temperature of the cord. A thorough examination of the observed temperature rise has indicated that transmission of nerve impulses across the synapses at the terminals of the sensory fibers is accompanied by generation of a considerable amount of heat in the substantia gelatinosa. This discovery of the "thermal response" of the spinal cord has given us a new, useful tool for studying the effects of various chemicals on synaptic transmission. We also examined excitation processes in the bullfrog retina by using our thermal detectors and piezoelectric sensors. We found that the photoreceptors in the dark-adapted retina are capable of releasing thermal energy which is more than one million times as large as the energy of the light pulse used for stimulation. Furthermore, we found it possible to analyze the processes of synaptic transmission in the retina by taking its mechanical responses as an index.

This is a continuation of Project #Z01 MH 00981 LNP.

Objectives: The objective of the present research is to elucidate the function of the nervous system by examining non-electrical manifestations of excitation processes. The major portion of our present-day knowledge of the function of the nervous system is derived from experimental findings obtained by measuring changes in electric potentials and currents in various parts of the system. The high sensitivity and the rapidity of response of various devices designed to record electrical events had led us to heavily rely on the results of analyses of the electrical signs of excitation processes. In recent years, however, it has become possible to record optical, mechanical and thermal changes in the nervous system with a reasonably high time resolution. During the past years, the sensitivity and the response-time of the thermal detectors we have constructed are greatly improved. Consequently, we expect that our investigation of functions of the nervous system by use of our optical, thermal and mechanical devices continues to unravel new phenomena in the nervous system.

Methods: Piezoelectric sensors are employed for measuring rapid changes in the tension or pressure in the nervous system. Bifurcated light guides are used for detecting changes in the turbidity. Thin sheets of polyvinylidene fluoride--synthetic pyroelectric material--are employed to construct heat sensors with a high sensitivity and a short response time. Quite recently, we have constructed a sensitive heat sensor which has a very small effective surface.

Major Findings:

(1) Detection of heat production associated with synaptic transmission.

Using bullfrog spinal cord preparations, we found that electric stimulation of the dorsal roots evokes a rapid rise in the rate of heat production by the cord. Immersion of the cord in a salt solution containing a low calcium and high magnesium ion concentration was found to suppress the observed "thermal response" (i.e. the transient rise of in heat production evoked by stimulation). Strong electric shocks applied to the ventral roots did not evoke any thermal response. The observed amplitude of the thermal response was enhanced considerably when the spinal cord was hemisected horizontally and the cut surface of the dorsal half-cord was brought in contact with the heat sensor. This and other tests have clearly indicated that the observed heat production is associated with synaptic transmission at the afferent fiber terminals. During the past three months, we have carried out a quantitative analysis of this newly discovered phenomenon. A study of the effects of various neuropharmacological agents on the synaptic heat is now in progress. In a preliminary experiment, we have demonstrated synaptic heat in the spinal cord of the new-born rat.

(2) Heat generated by bullfrog photoreceptors.

Last year, we found that the photoreceptors in the bullfrog retina respond to brief light pulses with a rapid generation of heat. This year, we analyzed properties of the thermal responses of the photoreceptor cells in detail by using greatly improved heat sensors. We found that, at the level of light intensity which delivers roughly one photon per rod, the energy released by the photoreceptors (in the form of heat) is more than one million times as large as the energy absorbed by the receptors. The significance of this finding is not altogether clear at present.

(3) Study of spread of excitation processes in the retina.

By taking mechanical responses of the isolated bullfrog retina as an index, the sequence of spread of excitation processes from the photoreceptor cells to the ganglion cells was analyzed. The time course of the force developed by the retina was explained by comparing the sequence of force development with that of the electro-retinogram. We came to the conclusion that the nerve cells in the retina swell during depolarization and shrink during hyperpolarization.

Significance to Biomedical Research:

Our knowledge of the function of the vertebrate nervous system is at present quite limited. There are inconsistencies in the present-day interpretation of the effects of drugs on the spinal cord. Studies of non-electrical manifestations of excitation processes are expected to lead us to a better understanding of the normal, as well as abnormal, function of the nervous system.

Proposed Course of Project:

We have just started investigating the effects of various chemical agents on the bullfrog spinal cord by taking heat production as an index. We are planning to apply our technique of recording non-electric signs of excitation processes to the vertebrate cerebrum.

Publications:

Tasaki, I., and Byrne, P.M.: Heat production associated with synaptic transmission in the bullfrog spinal cord. Brain Res. 407: 386-389, 1987.

Tasaki, I.: On the mechanism of hypersensitivity in nerve fibers and cells. In Chalazonitis, N., and Gola, M. (Eds.): Inactivation of Hypersensitive Neurons. New York, Alan R. Liss, Inc., 1987, pp. 311-319.

Tasaki, I., and Byrne, P.M.: Rapid mechanical changes in the amphibian retina evoked by brief light pulses. Biochem.

Biophys. Res. Commun. 143 (1): 93-97, 1987.

Tasaki, I., Byrne, P.M., and Masumura, M.: Detection of thermal responses of the retina by use of polyvinylidene fluoride multilayer detector. Japan. J. Physiol. (in press), 1987.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00881-31 LCM

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Intermediary Energy Metabolism in Mammalian Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Elaine E. Kaufman Research Chemist LCM, NIMH

Others: Thomas Nelson Medical Officer (Research) LCM, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.7

PROFESSIONAL:

1.2

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This report will describe work carried out in this laboratory which resulted in the discovery and characterization of a mammalian mitochondrial transhydrogenase, an enzyme capable of transferring hydrogens from specific hydroxyacids to equally specific ketoacids. This work suggests new catabolic pathways for L- β -hydroxybutyrate, γ -hydroxybutyrate and α -ketoglutarate and may explain the origin of the elevated levels of certain hydroxyacids found in patients with specific organic acidemias.

Project Description:Objectives:

The objectives of this study has been the isolation, characterization and purification of a mitochondrial hydroxyacid-oxoacid transhydrogenase found in brain, liver and kidney. The role of this transhydrogenase in the metabolism of such key metabolic intermediates as L- β -hydroxybutyrate, and α -hydroxyglutarate and γ -hydroxybutyrate is also of interest.

Methods:

The mitochondrial transhydrogenase described in this report has been purified using the following techniques: 1) differential centrifugation, 2) salt fractionation, and 3) column chromatography. The products of the reaction have been identified using gas-liquid chromatography either alone or in combination with mass spectroscopy, paper and thin layer chromatography. Subcellular localization and quantification of one of the enzymes has been confirmed by antibody titration. Enzyme kinetics have, in general, been carried out using a spectrophotometric method.

A mitochondrial hydroxyacid-oxoacid transhydrogenase was discovered when experiments with an antibody to the purified cytosolic γ -hydroxybutyrate dehydrogenase indicated that there was a second enzyme which could catalyze the oxidation of the hydroxy-acid, γ -hydroxybutyrate. This new enzyme was located in the mitochondria fraction isolated from brain, liver, and kidney and, moreover, did not require added NAD^+ or NADH. The oxidation of the hydroxyacid did, however, have an absolute requirement for α -ketoglutarate. In contrast to the well known α -ketoglutarate requiring dioxygenases the reaction catalyzed by this enzyme did not require molecular oxygen and resulted in the formation of α -hydroxyglutarate.

We later found that several other oxoacids, pyruvate, oxolactate and α -keto adipate could substitute for α -ketoglutarate but that α -ketoglutarate is the preferred substrate. The hydroxyacids which we have so far found to be substrates for this enzyme are L- β -hydroxybutyrate, γ -hydroxybutyrate and D- α -hydroxyglutarate.

Inasmuch as no added cofactor is required by this enzyme, we assume that the enzyme contains a tightly bound cofactor, possibly NAD^+ , NADH, which is oxidized and reduced on the protein. Proof of this must await purification of sufficient quantities of the protein to identify the cofactor.

In summary, a new enzyme has been isolated which can catalyze transhydrogenation reactions involving specific hydroxyacids and equally specific oxoacids. This is the first report of a mammalian enzyme capable of oxidizing L- β -hydroxybutyrate.

Significance to Biomedical Research and to the Program of the Institute:

A completely new enzyme which can catalyze the transfer of hydrogens from hydroxyacids to oxoacids has been isolated. The discovery of this enzyme may

provide new clues to the origin of specific hydroxyacids such as the 2-hydroxyglutaric acid found in the inherited metabolic aciduria, glutaric aciduria type II, a condition which leads to both peripheral and central nervous system disorders.

Proposed Course:

Aspects of this project which deal specifically with the metabolism and mode of action of γ -hydroxybutyrate have been completed and are being prepared for publication as is the work described above on the mitochondrial transhydrogenase.

A new initiative which will involve an investigation of some of the metabolic interrelationships of glia and neurons, in particular those for which GABA and glutamate are the predominate neurotransmitter, will be started. It is anticipated that these studies will utilize freshly isolated tissue, cells in culture as well as brain dialysis techniques.

Publications:

Kaufman, E. and Nelson, T.: Evidence for the participation of a cytosolic NADP^+ -dependent oxidoreductase in the catabolism of γ -hydroxybutyrate in vivo. J. Neurochem. 48: 1935-1941, 1987.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00882-20 LCM

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on Regional Cerebral Circulation and Metabolism

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I.: Louis Sokoloff Chief, Lab. Cerebral Metabolism LCM, NIMH

Others: Charles Kennedy Guest Researcher LCM, NIMH
 Thomas Nelson Medical Officer (Research) LCM, NIMH
 Carolyn B. Smith Research Chemist LCM, NIMH
 Gerald A. Dienel Senior Staff Fellow LCM, NIMH
 Nancy Cruz Biologist LCM, NIMH
 Nancy Eng Chemist LCM, NIMH

COOPERATING UNITS (if any)

Theoretical Statistics & Mathematics Branch, NIMH (C.S. Patlak & K.D. Pettigrew);
 NINCDS, NIH (I. Kopin); NIDA, ARC, Baltimore, Maryland (L. Porrino).

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

9.50

PROFESSIONAL:

6.00

OTHER:

3.50

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The deoxyglucose method for the quantitative determination of rates of local glucose consumption in the discrete functional and structural components of the brain of conscious or anesthetized laboratory animals was developed in this laboratory over 10 years ago. In this method [¹⁴C]deoxyglucose is employed as a tracer for glucose flux through the hexokinase step; the product, [¹⁴C]deoxyglucose-6-phosphate, is measured by quantitative autoradiography. The method continues to be used to study alterations in local energy metabolism in a variety of physiological, pharmacological and a limited number of pathological states. Its suitability to a wider range of pathologic conditions is being extended and special time constraints which may be present in the method's adaptation for use in human subjects with [¹⁸C]fluorodeoxyglucose and PET have been examined.

OTHER INVESTIGATORS (CONTINUED)

Kathleen Schmidt	Computer Systems Analyst	LCM, NIMH
Victor Ho	Guest Researcher (Hughes Scholar)	LCM, NIMH
Giovanni Lucignani	Guest Researcher	LCM, NIMH
Therese M. Jay	Visiting Fellow	LCM, NIMH
Kentaro Mori	Visiting Fellow	LCM, NIMH
Quang Vo	Computer Programmer	LCM, NIMH
Ernesta Palombo	Visiting Fellow	LCM, NIMH
Hajime Nakanishi	Visiting Fellow	LCM, NIMH

Project Description:

The deoxyglucose method, both in its original form and in its adaptation for use in human subjects, has been widely used by investigators throughout the world for over a decade. It has also been employed by members of this laboratory in the study of a variety of physiological conditions as reported previously and as given below. In the interests of extending the method's general applicability, the Laboratory has refined the model on which the method is based. This revision takes into account new information on the intracellular sites of phosphatase activity, and thereby defines more accurately the late time course when radioactive label is lost from the tissue. The new model provides the basis for understanding the time limits after administration of the labeled deoxyglucose during which valid measurements can be made, and to correct for processes that become significant with the long scan times required when PET is employed. Also many of the experiments planned over a year ago to permit the deoxyglucose method to be extended for use in a wide range of pathophysiological states have been successfully accomplished, and work done in response to criticisms by others has been concluded. These diverse studies related to the deoxyglucose method are separately described below.

I. APPLICATIONS OF THE DEOXYGLUCOSE METHOD

- A. Dr. Linda Porrino (NIDA) and Dr. Ernesta Palombo, in collaboration with Drs. Irwin Kopin and Krystof Bankiewicz of NINCDS, have extended their studies with the deoxyglucose method applied to the Parkinson's syndrome induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

Objectives:

- 1) To measure glucose utilization in the neural pathways involved in the production of abnormal motor function in the monkey with hemiparkinsonism and to map the circuits involved in the therapeutic administration of L-DOPA.
- 2) To study the acute effects of MPTP on glucose utilization in monkeys and their prevention by pretreatment with MAO-inhibitor drugs.

Major Findings:

In the animals with hemiparkinsonism decreases in glucose utilization were found in the external segment of the globus pallidus ipsilateral to the side of neuro- toxin administration while decreases were found bilaterally in the substantia nigra and in the subthalamic nuclei. Treatment with L-DOPA restored normal rates of glucose utilization in the subthalamic nucleus and in the lateral substantia nigra while resulting in rates above normal in the substantia nigra reticulata and in the internal segment of the globus pallidus. The findings in hemiparkinsonian monkeys in the visual system and pathways concerned with eye movements have been incorporated in a manuscript now in press.

Acute administration of MPTP resulted in marked increases in glucose utilization in the substantia nigra pars compacta and portions of the ventral tegmental area, along with severe metabolic depression in virtually all other structures of the brain. Pretreatment with the MAO-inhibitor, pargyline, which protects against the long term toxicity in the nigra, also prevents the acute increases in the nigra and ventral tegmental area.

- B. Dr. Therese Jay completed her studies of local cerebral blood flow in the mouse. While these left unexplained the reason for the relatively high rate of metabolism of the fasciculus retroflexus in the normal mouse, they served to provide the methodology for local cerebral blood flow in this species. The report of this work is now in press.
- C. Drs. Kentaro Mori, Hajime Nakanishi, and Charles Kennedy, in collaboration with Dr. Kenneth Kellar of the Department of Pharmacology of Georgetown University School of Medicine, are completing the study of the local metabolic effects of phencyclidine, the agent now prominent because of its mind-altering effects and abuse among young people. Detailed analysis of the autoradiographs has lagged because of limited availability of personnel and computer times, but it is under high priority examination.

Objectives:

To correlate the known psychological and behavioral effects of phencyclidine with the effects on local cerebral glucose utilization in the rat over a wide range of doses.

Major Findings:

Selective reductions in the rates of metabolism were found in primary sensory pathways, such as auditory and visual pathways, while enormous increases were observed in limbic structures, such as the amygdala, hippocampus, cingulate gyrus, etc. The work is in progress, but it already demonstrates that limbic seizure activity may be a major consequence of PCP use and the basis of the violent and aggressive behavior associated with the use of this drug.

- D. Drs. Therese Jay and Charles Kennedy, in collaboration with Dr. Robert Abrams of the Department of Obstetrics and Gynecology at the University of Florida in Gainesville, concluded their study of local metabolic rate of the brain during rapid-eye-movement sleep. The results showed diffuse increases in local cerebral glucose utilization throughout the brain during REM sleep. The manuscript reporting this work has been submitted for publication.
- E. Dr. Linda Porrino concluded her evaluation of the effects of cocaine on cerebral metabolism, a project initiated jointly with Dr. Floyd R. Domer in the previous year.

II. EXTENSION OF THE DEOXYGLUCOSE MODEL TO A 5-PARAMETER MODEL

The deoxyglucose method assumes that products of deoxyglucose phosphorylation are trapped in the tissues for the duration of the experimental period. In its application no detectable loss is found during the first 45 minutes, a small loss occurs at 60 minutes, and progressively greater losses occur at 90 and 120 minutes following an intravenous pulse of [^{14}C]deoxyglucose. The loss, when it occurs, is presumably due to the action of glucose-6-phosphatase. Fishman and Karnovsky (J. Neurochem. 46: 371, 1986) have explained the lag in the appearance of the phosphatase activity by finding that the hydrolysis is rate-limited by the diffusion of deoxyglucose-6-phosphate from the cytosol, where it is formed, across the endoplasmic reticular membrane to the site where glucose-6-phosphatase resides. The original deoxyglucose model was, therefore, revised to include this new information, and an equation was derived to describe the kinetic behavior of labeled deoxyglucose and deoxyglucose-6-phosphate in the tissue in the presence of these constraints. The new equation contains 5 rate constants, including one for the diffusion of deoxyglucose-6-phosphate across the endoplasmic reticular membrane and another for its hydrolysis by glucose-6-phosphatase.

Drs. Giovanni Lucignani, Kentaro Mori, Therese Jay, Ernesta Palombo, Thomas Nelson and Ms. Kathleen Schmidt carried out the necessary experiments to obtain estimates of the values of these rate constants. These experiments provided the time courses of arterial plasma and tissue concentrations of ^{14}C over a period from 2.5-120 minutes. The new equation was fit to the measured data by a non-linear least-squares routine to obtain the best fitting rate constants. The data were also analyzed by the graphical evaluation technique which provides an estimate of the time when loss of product begins to take place. These studies have been presented at an international meeting, and a manuscript on this work is in preparation.

III. ADAPTATION OF THE DEOXYGLUCOSE METHOD FOR USE IN PATHOPHYSIOLOGICAL CONDITIONS

The original deoxyglucose method was designed for use in laboratory animals under physiologic conditions. When normal physiological limits are exceeded, as may occur during status epilepticus, ischemia, or severe hypoglycemia, the lumped constant of the operational equation is altered. In order to make

possible the application of the deoxyglucose method to such pathophysiological conditions it is planned to develop a procedure to obtain values for the local lumped constant. Through the use of [^{14}C]methylglucose it is now possible to measure local glucose concentration in the brain. This, in turn, makes possible an estimate of the ratio of the distribution volumes for glucose and deoxyglucose in local brain regions and, therefore, of the lumped constant itself. The experiments require the preparation of animals in which various, specified plasma concentrations of glucose over a wide range can be induced and maintained for long periods. An equation and programmed infusion schedule for this purpose has now been refined and tested with success by Dr. Kentaro Mori. The complex experiments in which [^3H]deoxyglucose or [^{14}C]methylglucose and glucose concentrations in local regions of brain will be measured simultaneously in the same animal under steady state conditions have begun. Others participating in these experiments are Drs. Gerald Dienel, Thomas Nelson, Therese Jay, Ernesta Palombo, Charles Kennedy, Carolyn Smith, and Ms. Nancy Cruz.

Objectives:

To make possible the determination of the local lumped constant in the brains of animals under pathophysiological conditions. This determination of glucose utilization in all local subdivisions of brain could be reliably measured in pathophysiological states.

Major Findings:

The large number of complex experiments involving the creation of a steady state at specified plasma glucose concentrations and, simultaneously, a steady state for plasma deoxyglucose or methylglucose, have been completed. These experiments, terminated with a procedure for instantaneous freezing of the brain, and followed by analyses of plasma and tissue for their concentrations of glucose, deoxyglucose or methylglucose, have permitted the calculation of a family of curves describing distribution volumes over a wide range of plasma glucose concentrations. These fundamental studies are preliminary to the conduct of a planned group of experiments involving double label isotopes for the autoradiographic measurement of brain concentrations of glucose and deoxyglucose in the same animal. Information from the combined groups of experiments will make possible the calculation of the local lumped constant in such conditions as ischemia, sustained seizures, and cerebrovascular disorders.

IV. WORK IN RESPONSE TO PAPERS CRITICAL OF THE DEOXYGLUCOSE METHOD

The laboratory has largely completed the time-consuming projects of responding to criticisms of the deoxyglucose method a year ago. The claims of Huang and Veech that the activity of glucose-6-phosphatase in brain is very active (J. Biol. Chem. 257: 11358-11363, 1982) were shown to be without foundation and due to inadequate purification procedures. It remained for Dr. Gerald Dienel to identify the major sources of contaminating compounds in the brain glucose fraction of Huang and Veech. He has shown these to consist of at least 7 amino acids in addition to glucosamine. Furthermore, Dr. Dienel showed that the

glucose purification procedures employed by Huang and Veech not only failed to purify the radioactive glucose but actually provided an environment for detritiation of the labeled glucose and for side reactions that converted glucose and contaminants to other ^{14}C labeled compounds. The improperly purified glucose fraction was responsible for the erroneous conclusions of Huang and Veech. One more paper by G. Dienel describing these studies is close to final form for submission and publication.

Significance to Biomedical Research and to the Program of the Institute:

The deoxyglucose method has made it possible for the first time to measure the rates of glucose utilization simultaneously in all functional and structural components of the central nervous system of conscious, behaving animals and now also in man. Because the method was developed in our Laboratory, it has been our responsibility to survey its applicability to the various types of conditions in which it might be applicable. The program has, therefore, been somewhat heterogeneous covering a wide range of physiological, pharmacological, pathological, and altered behavioral states. The method and its wide-ranging usefulness have now been more or less established, and it is used extensively throughout the world in neuroanatomical, neurophysiological, neuropharmacological, psychiatric, neurological, and neurosurgical research. Its wide acceptance is directly related to the results of studies in this project.

Proposed Course:

Applications of the deoxyglucose method to problems of neurophysiology, neuropharmacology, neurology, and psychiatry will be continued. A project has been initiated and will be continued to adapt the method for use in neuropathological conditions such as stroke, status epilepticus, etc. Efforts will be made to improve the quantitative resolution of the method to the single cell and subcellular levels. Immunocytochemical techniques will be introduced with the aid of Dr. Bernard Driscoll to correlate local cerebral rates of glucose utilization with local levels of neuropeptides and the host of putative neurotransmitters and neuromodulators. A cell culture facility has been established in the Laboratory by Dr. B. Driscoll to allow studies of cellular mechanisms of carbohydrate transport across cell membranes which are necessary to define the rates of glucose utilization in neuronal and glial cellular components of the cerebral tissue.

Publications:

Sokoloff, L.: Basic principles in the imaging of rates of biochemical processes in vivo. In Hayaishi, O. (Eds.): Biomedical Imaging--From Anatomy to Physiology and Biochemistry. Tokyo, Japan, Academic Press, Japan, Inc., 1986, pp. 183-217.

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Porrino, L.J., Burns, R.S., Crane, A.M., Palombo, E., Kopin, I.J., and Sokoloff, L.: Changes in local cerebral glucose utilization associated with parkinson's syndrome induced by 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) in the primate. Life Sci. 40: 1657-1664, 1987.

Sokoloff, L.: Book Review. Radionuclide Imaging of the Brain. (Vol 1, Contemporary Issues in Nuclear Imaging. Edited by B. Leonard Holman. New York: Churchill Livingstone, 1985, 232 pg.). J. Neurosci. Res. 17: 199, 1987.

Lucignani, G., Namba, H., Nehlig, A., Porrino, L.J., Kennedy, C., and Sokoloff, L.: Effects of insulin on local cerebral glucose utilization in the rat. J. Cereb. Blood Flow & Metab. 7: 309-314, 1987.

Kadekaro, M., Vance, W. H., Terrell, M. L., Gary, Jr., H., Eisenberg, H.M., and Sokoloff, L.: Effects of antidromic stimulation of the ventral root on glucose utilization in the ventral horn of the spinal cord in the rat. Proc. Natl. Acad. Sci. USA 84: 5492-5495, 1987.

Porrino, L.J., Burns, R.S., Crane, A.M., Palombo, E., Kopin, I.J., and Sokoloff, L.: Local cerebral metabolic effects of L-DOPA therapy in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in monkeys. Proc. Natl. Acad. Sci. USA 84: 5995-5997, 1987.

Sokoloff, L.: Foreword. In Wood, J.H. (Ed.). Cerebral Blood Flow: Physiologic and Clinical Aspects. McGraw-Hill, 1987, (in press).

Ito, M., Kadekaro, M., and Sokoloff, L.: Local glucose utilization of the brain and pineal gland during stimulation of the cervical sympathetic trunk. J. Pineal Res. (in press) 1987.

Ho, V.W., Porrino, L.J., Crane, A.M., Kopin, I.J., and Sokoloff, L.: Alterations in local cerebral utilization in the oculomotor system of MPTP-induced Parkinsonian monkeys. Ann. Neurol. (in press) 1987.

Sokoloff, L., Kennedy, C., and Smith, C. B.: The [¹⁴C]deoxyglucose method for measurement of local cerebral glucose utilization. Neuromethods Vol. 15. Humana Press, 1987, (in press).

Sokoloff, L.: Circulation and energy metabolism of the brain. Basic Neurochem. 4th Ed.. Raven Press, 1987, (in press).

Sokoloff, L.: Basic principles in imaging of regional cerebral metabolic rates with radioisotopes. (Proceedings of the NATO ASI Meeting in L'Aquila, Italy, June, 1986) (in press).

Jay, T.M., Lucignani, G., Crane, A.M., Jehle, J., and Sokoloff, L.: Measurement of local cerebral blood flow with [¹⁴C]iodoantipyrine in the mouse. J. Cereb. Blood Flow & Metab. (in press) 1987.

Abrams, R.M., Hutchison, A.A., Jay, T.M., Sokoloff, L., and Kennedy, C.: Local cerebral glucose utilization nonselectively elevated in rapid eye movement sleep of the fetus. Brain Res. (in press) 1987.

Palombo, E., Porrino, L.J., Krzysztof, S., Bankiewicz, K.S., Kopin, I.J., and Sokoloff, L.: Comparison of acute and chronic effects of MPTP on local cerebral glucose utilization in monkeys. (International Symposium on Neurotoxicology, Turin, Italy, May 5-7, 1987) (in press) 1987.

Jay, T.M., Abrams, R.M., Hutchison, A.A., Kennedy, C., Schmidt, K., and Sokoloff, L.: Variations du debit sanguin et metabolisme cerebral au cours du sommeil. Cereb. Circ. & Metab. (in press) 1987.

Sokoloff, L.: Foreword to Proceedings of the Eric K. Fernstrom Symposium, Neural Regulation of Brain Circulation. (Proceedings of the Eric K. Fernstrom Symposium, Lund, Sweden, June 21-23, 1985), (in press) 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00887-10 LCM

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Extended Visual System of the Macaque Monkey

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Charles Kennedy Guest Researcher LCM, NIMH

Others: Louis Sokoloff Chief LCM, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.25

OTHER:

0.75

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although the project began with its focus on mapping the visually responsive cortical areas, the experiments also permit an analysis of the sensory-motor system. Normal control monkeys with their visual pathways intact responded to the visual cues by pressing a lever with one hand. This involved an asymmetrical pattern of local glucose utilization in brain involving a wide expanse of cortical and sub-cortical structures. An analysis of the pattern of asymmetry provides new information with respect to the localization sensory-motor function. The data obtained to date indicate that a much larger portion of brain regions are unilaterally activated on unimanual activity than has been appreciated previously.

Project Description:Objective:

To map all regions of monkey brain (motor and sensory) which are involved in the performance of a task involving the use of one hand.

Methods Employed:

Normal controls involved in continuous, unimanual lever pressing in response to visual cues were studied with the deoxyglucose method and provided an opportunity to study the localization of motor activity. To the extent that motor activity involving one arm and hand is accompanied by neuronal discharges in the contralateral hemisphere, a side to side comparison of metabolic rates in homologous regions provides a measure of the functional participation of the regions in the motor behavior. During such behavior there is, of course, a concomitant stimulation of a number of sensory modalities, eg. touch, pressure and joint position. The metabolic studies of the unimanually active monkeys therefore permitted a mapping of sensory pathways. The large task of making a detailed side to side comparison of the large number of potentially activated structures in the animals was begun only recently.

Major Findings:

In the study of local regions responsive to sensorimotor stimulation by unimanual lever pressing the analysis has indicated that the metabolically activated regions are widely distributed in both cortical and sub-cortical structures. Some structures, which might be expected to have large right-left differences in their metabolic rates on the basis of others' neurophysiologic observations, proved to have relatively small differences, eg. the globus pallidus. Conversely, surprisingly large differences were found in several cortical regions generally not regarded as being active during motor activity. Among these was the inferior bank of the superior parietal lobule. Lesion and behavioral-electrophysiological experiments indicate that many independent functional entities coexist in their representation in the superior parietal lobule. The deoxyglucose method as applied here appears to have localized with great precision the part of this diverse cortical region which contributes to visually directed movements of one arm and hand. Metabolic evidence of the participation of several other regions is demonstrated in these studies. The analysis of the data is continuing.

Significance to Biomedical Research and to Program of the Institute:

These studies add new information on the localization of functional activity in brain.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00889-08 LCM

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Method for the Determination of Local Rates of Protein Synthesis in Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.:	Carolyn B. Smith	Research Chemist	LCM, NIMH
Others:	Louis Sokoloff	Chief	LCM, NIMH
	Christina Eintrei	Visiting Fellow	LCM, NIMH
	Charles Kennedy	Guest Researcher	LCM, NIMH
	Mortimer Mishkin	Chief	LN, NIMH
	Richard Nakamura	Guest Researcher	LN, NIMH

COOPERATING UNITS (if any)

Department of Neurosurgery, Univ. of Texas (M. Kadekaro); Dept. of Obstetrics & Gynecology, Univ. of Florida (R. Abrams); Department of Neurosurgery, SUNY (D. Dow-Edwards)

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.8

PROFESSIONAL:

1.6

OTHER:

1.2

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A method has been developed for the estimation of local rates of protein synthesis in brain in vivo. The method is based on the use of L-[¹⁴C]leucine as a tracer for the incorporation of leucine into protein. Six kinetic models for the behavior of leucine in brain have been designed. By mathematical analysis of the kinetics of exchange of the amino acid between plasma and the tissue pool(s) and its incorporation into protein, equations have been derived for each model that define the rate of amino acid incorporation into protein in terms of the time course of plasma-specific activity, final tissue concentration of ¹⁴C, and experimentally determined kinetic constants. Tissue concentrations of ¹⁴C are determined by quantitative autoradiography. Experiments have been carried out to test the validity of the various models and to determine the kinetic constants to be used in the operational equation.

Studies of the effects of normal development, sleep, anesthesia, electrical stimulation and hypothyroidism on cerebral protein synthesis have been undertaken in order to examine the potential usefulness of the leucine method.

Project Description:Major Objectives:

The overall objectives of this research project are:

1. To develop a method for the measurement of local rates of protein synthesis in brain.
2. To test the usefulness of the method in the study of neurobiological problems.

A method is being developed for the estimation of local rates of protein synthesis in brain in vivo. This method is similar to the [^{14}C]deoxyglucose method in that it is based on enzyme kinetic principles as applied to the measurement of reaction rates in vivo with labeled tracers as substrates. The two requirements for this type of method are: 1) label must be primarily in the form of labeled product (labeled protein) at the end of the experiment, and 2) a model for the behavior of the precursor in brain in order to calculate the integrated specific activity of the precursor at the site of the reaction in terms of measurable variables. In order to satisfy the first requirement, [$1\text{-}^{14}\text{C}$]L-leucine has been chosen as the labeled tracer. The only metabolic pathway for leucine, apart from protein synthesis, entails a transamination followed by a decarboxylation. Therefore, in the metabolism of [$1\text{-}^{14}\text{C}$]leucine the label is transiently transferred to α -ketoisocaproic acid and ultimately to $^{14}\text{CO}_2$ which is then diluted in the large CO_2 pool in brain produced by carbohydrate metabolism. There are, therefore, no residual radioactive products other than labeled protein. In order to meet the second requirement we have designed kinetic models for the behavior of leucine in brain. All of these models are based on the following assumptions and requirements:

- 1) Homogeneous tissue compartments
- 2) No isotope effect
- 3) No release of labeled leucine from labeled protein during the 60-minute experimental period
- 4) Complete loss of label from metabolic degradation of leucine
- 5) Steady state for unlabeled leucine

We have developed six such models, and by mathematical analysis we have derived operational equations for all of them. The models are of progressively increasing complexity as regards the number of compartments and the interrelationships among them. Regardless of the degree of complexity of the model used, the operational equation has the same general form. The numerator is composed of the total amount of radioactivity in the tissue at the end of the experiment minus term(s) for the lag between the precursor pool and the plasma. Optimal design of the experimental procedure allows us to reduce this expression to the total radioactivity in the tissue divided by the integrated plasma specific activity. The terms in the numerator for the free leucine pools can be eliminated by fixing and washing tissue sections. The expression for the lag in the denominator can be made very small by administering a pulse of [^{14}C]leucine

and allowing 60 minutes, estimated to be more than 10 half-lives of the precursor pool in white matter, for clearance of the free pool.

The specific aims pursued during this fiscal year were:

1. To determine the relative contribution of plasma leucine to the precursor pool for protein synthesis, and
2. To apply the [^{14}C]leucine method to the study of development and other neurobiological problems.

Methods Employed:

1. The determination of the relative contribution of plasma leucine to the precursor pool for protein synthesis.

The experiment consists of the determination of the specific activity of brain leucyl-tRNA and plasma leucine in a rat in a steady state for both labeled and unlabeled leucine in the plasma. If all of the leucine is derived from the plasma, the specific activity of the leucyl-tRNA will eventually reach that of the plasma. If there is a significant contribution of leucine from protein degradation, the ratio of the specific activities of leucyl-tRNA and plasma leucine will be a measure of the fractional contribution from plasma. A programmed intravenous infusion schedule for [^3H]leucine was designed that would produce and maintain a constant concentration of [^3H]leucine in the arterial plasma. This input function was obtained from a Laplace transform of the relationship between a pulse input of [^3H]leucine and the multiexponential output. The controlled infusion was achieved by means of a programmable infusion pump. Rats were maintained under a steady state for both labeled and unlabeled leucine for at least 30 minutes at which time they were guillotined and the brains and livers were rapidly removed. Brain and liver tRNA was separated and purified by differential centrifugation, and acid and phenol extraction techniques. Purified tRNA was deacylated at pH 10 and the amino acids were separated from the RNA by ethanol precipitation. The specific activity of the leucine in this amino acid fraction and the specific activity of the plasma samples were determined as follows: The amino acids were derivatized with [^{14}C]dansyl chloride. The dansylated neutral amino acids were separated from other products of the dansylation reaction by thin-layer chromatography. Dansyl leucine was separated from other dansylated neutral amino acids by HPLC with a C18 column. The dansyl leucine peak was collected and counted for both ^{14}C and ^3H . The ratio of ^3H to ^{14}C is a measure of the specific activity of the leucine.

2. Studies of neurobiological problems.

Local rates of cerebral protein synthesis were determined with the [^{14}C]leucine method (Smith et al., J. Neurosci. 4: 2489-2496, 1984).

Major Findings:

1. The determination of the relative contribution of plasma leucine to the precursor pool for protein synthesis.

Results obtained in 6 experiments show that in brain 50-60% of the leucine pool which serves as the precursor pool for protein synthesis is derived from the plasma whereas in liver about 40% of the tRNA leucine pool is derived from the plasma. These results indicate that protein turnover will have to be taken into consideration in future studies of protein synthesis. This is the first time that the relative contribution of the plasma leucine to the tRNA amino acid pool has been determined in either brain or liver *in vivo*. Because these results are of interest in and of themselves they will be reported separately.

2. Studies of neurobiological problems.

In order to examine the potential usefulness of the leucine method, studies of neurobiological problems are being pursued with the assumption that the relative contribution of plasma leucine to the precursor pool for protein synthesis does not change with the experimental conditions. We have determined local rates of protein synthesis in normal, conscious male rats with Dr. Charles Kennedy. The course of normal development in Rhesus monkeys is also being examined. In addition, studies of slow wave sleep in Rhesus monkeys have been undertaken. Dr. Eintrei is investigating the effects of anesthesia on local rates of protein synthesis in rats. Experiments have been carried out on animals under either light barbiturate anesthesia or ketamine anesthesia. The autoradiographic results of these experiments are now being analyzed. Studies have been carried out in collaboration with Dr. Kadekaro at the University of Texas on the effects of repetitive stimulation of the sciatic nerve on local rates of protein synthesis in the spinal cord and the dorsal root ganglia. The autoradiographic results of these experiments are being analyzed. Studies of the effects of thyroidectomy in infant rats on cerebral protein synthesis have been carried out with Dr. Dow-Edwards at SUNY. The results of these experiments are being analyzed. A collaboration effort with Dr. Robert Abrams at the University of Florida to study the developmental time course of protein synthesis in fetal sheep brain has been initiated. Cerebral protein synthesis rates measured in two fetal sheep at 130 days gestation are exceedingly high (range 15-25 moles leucine/g/min).

Significance to Biomedical Research and Program of the Institute:

Protein synthesis is probably the most important biochemical process underlying the development, maturation, plasticity, maintenance, and long-term regulation of the nature and degree of functional activity of the nervous system. The structural, functional, and metabolic properties of the tissues largely reflect the role of structural and enzymatic proteins. Peptides that are considered to be neurotransmitters are in some, and possibly all, cases derived from the cleavage of large parent protein molecules. Many hormones within and outside the nervous system are proteins. It is, therefore, certain that changes in protein synthesis can and do alter function and that some mental and neurological dysfunctions reflect disturbances in this vital biochemical process.

This research is directed at determining the rates of protein synthesis in specific regions of the central nervous system with an ultimate resolution down to the cellular level. This provides for the first time the opportunity to study at the individual structural or anatomical level the changes in protein synthesis that may be the causes, consequences, or correlates of normal conditions, such as maturation, plasticity, differentiation, sleep, learning and memory, behavioral patterns, etc., or pathological conditions, such as hormonal disorders, aging, regeneration in response to injury, convulsive disorders, coma, etc.

Proposed Course:

Additional experiments will be carried out in order to determine by an independent analytical method the relative contribution of plasma leucine to the brain t-RNA pool. The Laboratory has just purchased a new Beckman amino acid analyzer which has the capability of detecting picomolar levels of amino acids. With the assistance of Gladys Deibler we are setting up this instrument to separate and detect the tRNA pool amino acids derived from rat brains in a steady state for both labeled and unlabeled leucine.

Applications of the [^{14}C]leucine method to studies of the effects of anesthesia on nerve stimulation, sleep, development, and hypothyroidism will be continued. Studies of the effects of testosterone on regeneration will be carried out in collaboration with Dr. Amy Yu, City University of New York Medical School.

Publications:

Holcomb, H.H., Links, J., Smith, C., and Wong, D.: Positron emission tomography: Measuring the metabolic and neurochemical characteristics of the living human nervous system. In Andreasen, N.C. (Ed.): Brain Imaging: Implications for Psychiatry. Washington, D.C., Amer. Psychiatric Press, 1987, (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00903-10 LCM
PERIOD COVERED October 1, 1986 to September 30, 1987		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Purification and Identification of Brain Proteinases and their Cleavage Products		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.:	Gladys E. Deibler	Research Chemist LCM, NIMH
Others:	Marian W. Kies Audrey Stone	Chemist Guest Researcher LCM, NIMH LDMI, NICHHH
COOPERATING UNITS (if any) Multiple Sclerosis Research Center; Dept. of Neurology, Georgetown University (J. Richert)		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Developmental Neurochemistry		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2.5	PROFESSIONAL: 1.5	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Our exhaustive review of the many papers on brain proteinases led us to choose <u>calcium activated neutral proteinases (CANP I, II and III)</u> as the brain enzymes that we will investigate. CANP I is located in the neurons, CANP II is located in the glia cells, and CANP III has not been associated with a particular structure. Presently, we are developing the specific analytical methods needed to extract, purify, characterize and assay the CANP's. Our previous investigation of human myelin basic protein (HBP) led to the identification and characterization of a new form of HBP with a molecular weight of 17.2 kDa and highly purified HBP-component 1, the unmodified 18.5 kDa protein. Highly purified HBP-component 1 was cleaved with thrombin and the resulting two peptides (1-97 & 98-170) were purified and characterized. HBP-component 1, 17.2 kDa HBP and the <u>two thrombic peptides (residues 1-97 & 98-170)</u> were used in collaboration with Dr. John Richert, Dept. of Neurology, Georgetown University. Forty <u>myelin basic protein-reactive T cell clones</u> were isolated from a multiple sclerosis patient and used to identify <u>human T cell recognition sites</u> on the HBP molecule. At least three sites have been identified: One in the N-terminal half of the molecule (residues 1-97), one in the C-terminal (residues 98-170), and one which spans residues 97-98. In collaboration with Dr. Audrey Stone, we have completed the investigation on the role of phosphorylation on the <u>conformational adaptability of bovine myelin basic protein (MBP)</u> . The limited digestion of MBP-components 2 + 3 yielded a mono-phosphorylated component which was phosphorylated only at threonine 97. From the circular dichroism of this <u>homogeneous mono-phosphorylated MBP</u> , we determined that the <u>β-structure</u> of this protein was increased by 7%, when compared with MBP-component 1. The single phosphorylation on threonine 97 produced a change in the macrostructure of the protein involving about 12 amino acid residues.		

Project Description:Objectives:

The completion of the determination of the T cell active sites of human myelin basic protein.

The isolation and purification of the calcium activated neutral proteinases and a study of their bond specificity with known CNS proteins and/or peptides as substrates.

Methods Employed:

Ion-exchange, affinity and size-exclusion chromatography, FPLC, HPLC, amino acid analysis and polyacrylamide slab gel electrophoresis were used in all our previous studies and will be used in our new project. A mini slab SDS polyacrylamide electrophoresis method and the Bradford Protein Assay have been developed for our new project. HPLC procedures are being investigated for the identification of digestion products of CANP.

Methods and programs for the identification and quantitation of methionine sulfoxide, methionine sulfone, $N^{G_N}G'$ dimethylarginine and monomethylarginine are being developed for our new 7300 Beckman amino acid analyzer.

Major Findings:

Our discovery that thrombin will not cleave the bond between arginine-98 and threonine-97, when the latter is phosphorylated, made it possible for us to obtain a homogeneous mono-phosphorylated MBP-component 3. The analysis of the CD spectra of this component, and components 1 and 2 indicates that the phosphorylation of threonine-97 promoted a 7% increase in the β -structure of the polypeptide chain.

The isolation of T cell clones reactive to HBP from a patient with multiple sclerosis proves the relevance of HBP as a potential autoimmune target in human demyelinating disease. Analysis of reactive T cell clones revealed at least three different antigenic determinants: one in the N-terminal (1-97) half of the molecule, one in the C-terminal (98-170) and one which spans residues 97-98. More clones were reactive to the C-terminal half of the molecule. All clones were reactive to the 17.2 kDa HBP. This means that the deletion of Exon 5 (residues 106-116) was not a part of an antigenic site. This is the first successful cloning of BP-reactive cells specific to different regions of the molecule.

Significance to Biomedical Research and the Program of the Institute:

The forty myelin basic protein (HBP)-reactive clones will be useful for both the further delineation of the human T cell recognition sites on HBP and the generation of anticonotypic monoclonal antibodies which might be used in the determination of the active site in a demyelinating disease.

Phosphorylation of threonine-97 has been shown to increase the ordered macrostructure of bovine myelin basic protein. These data may contribute to

understanding the role of phosphorylation of MBP in the development and stabilization of the myelin sheath.

Proposed Course:

The continued collaboration with Dr. John Richert to determine all of the HBP reactive T cell sites.

The extraction, purification and characterization of CANP I, II, III and the determination of their cleavage sites in brain proteins.

Publications:

Kira, J., Bacon, M.L., Martenson, R.E., Deibler, G.E., Kies, M.W., and Alvord, E.C., Jr.: Experimental allergic encephalomyelitis in rabbits. A major encephalitogenic determinant within residues 1-44 of myelin basic protein. J. Neuroimmunol. 12: 183-193, 1986.

Alvord, E.C., Jr., Hruby, S., Martenson, R.E., Deibler, G.E., and Law, M.J.: Evidence for specific polypeptide chain folding in myelin basic protein from reactions between fragments of the protein and monoclonal antibodies. J. Neurochem. 47: 764-771, 1986.

Deibler, G.E., Krutzsch, H.C., and Kies, M.W.: A new form of myelin basic protein found in human central myelin. J. Neurochem. 47: 1219-1225, 1986.

Richert, J.R., Rueben-Burnside, C.A., Deibler, G.E., and Kies, M.W.: Peptide specificities of myelin basic protein-reactive human T cell clones. Neurology, in press, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02216-04 LCM

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolic Mapping of the Brain during Rewarding Self-Stimulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Linda J. Porrino Guest Researcher LCM, NIMH

Others: Louis Sokoloff Chief LCM, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.00

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The deoxyglucose method is being used to study alterations in local cerebral glucose utilization resulting from rewarding electrical brain self-stimulation to discrete brain sites as well as resulting from the administration of drugs of abuse. By mapping metabolic activity in rats under these conditions, information can be obtained about those areas of the brain involved in motivation and reinforcement.

Project Description:Objectives:

The goal of the present project is to map those regions of the rat brain activated during positive reinforcement processes. Both rewarding electrical brain stimulation and the administration of psychostimulants known to produce rewarding effects are being studied.

Methods Employed:

The 2-[¹⁴C]deoxyglucose method affords a novel and unique opportunity to map functional neural pathways simultaneously in all anatomical components of the central nervous system. This method, therefore, allows the identification of complex neural circuits that are functionally active during various behavioral and pharmacological manipulations.

The goal of the present project is to map those regions of the rat brain activated during positive reinforcement processes. Both rewarding electrical brain stimulation and the administration of psychostimulants known to produce rewarding effects are being studied.

Major Findings:

The results of previous experiments in this series indicate an involvement of the nucleus accumbens, medial thalamus and prefrontal cortex in reinforcement. These data, however, are confounded to a degree by differences in the motor behavior of the compared groups - i.e. lever-pressing vs. sitting quietly. In order to eliminate the motor component and to distinguish better between motor and reinforcement effects, the following experiment was conducted. The standard protocol for the 2-DG method was applied to four groups: 1) intracranial self-stimulation (ICSS) prior to 2-DG in stimulation chamber, 2) experimenter administered stimulation prior to 2-DG in stimulation chamber, 3) no stimulation prior to 2-DG in same chamber, and 4) ICSS prior to 2-DG in home cage. All animals received no stimulation for the 2 sessions just prior to and during the 2-DG procedure. Since the animals in all groups were stimulated during the measurement of glucose utilization, the only difference between the groups was their stimulation history.

Preliminary analysis of these data indicates that in the large majority of brain regions, rates of glucose utilization were identical, a similarity that can be considered a reflection of the similarity in the behavioral states of the animals in all groups. There were, however, differences in a small number of structures, the prefrontal cortex and nucleus accumbens, in which rates of energy metabolism were higher in the ICSS group tested in the chamber in which they had had ICSS experience than rates of rats in other groups. These differences cannot be attributed to stimulation effects, as groups 2 and 4 also received similar amounts of electrical stimulation, either ICSS or experimenter-administered. Although these results are highly preliminary, they suggest that these areas are important for the expression of conditioned positive reinforcement.

Proposed Course:

The application of the 2-deoxyglucose method to studies of reinforced behavior will be extended to include behavior reinforced by other reinforcers other than drugs and electrical stimulation. The effects of the administration of other drugs of abuse that are considered highly rewarding such as phencyclidines is in progress.

Publications:

Porrino, L.J.: Using the quantitative 2-[¹⁴C]deoxyglucose method for metabolic mapping of the brain during reinforced behavior. In Dahlstrom, A. (Ed.): (Proceedings of the VI International Catecholamine Symposium, Jerusalem, Israel, June 14-19, 1987), (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02217-04 LCM

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Plasticity in the Developing Monkey Visual System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Carolyn B. Smith Research Chemist LCM, NIMH

Others: Louis Sokoloff Chief LCM, NIMH

 Karen D. Pettigrew Res. Math. Statistician TSMB, NIMH

 Susan Herdman Guest Researcher LCM, NIMH

COOPERATING UNITS (if any)

Department of Neurology, Johns Hopkins Medical School, Baltimore, MD (R. Tusa)

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.8

PROFESSIONAL:

0.5

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The postnatal development of the central visual pathways depends on the quality of the visual environment. During the critical period in the primate visual system environmental manipulation can modify the physiological properties of visual cortical cells. The purpose of this project is to study the underlying biochemical events that imbue the nervous system with the property of plasticity. Protein synthesis is a biochemical process which is involved in bringing about changes in morphology, adjustments in growth rates, and remodeling and maintenance of structures. We have, therefore, used the [¹⁴C]leucine method to study the relationships between local plastic changes which occur in the developing monkey visual system and local rates of protein synthesis.

Project Description:Objectives:

The purpose of this project is to study the biochemical events associated with plasticity. The developing rhesus monkey visual system has been chosen as a model system because the physiological and anatomical responses to deprivation have been so well described by others. We have focussed initially on the process of protein synthesis because it is a requirement for growth and development and because changes in morphology and rates of growth and remodeling and even maintenance of existing structures should be reflected in changes in rates of protein synthesis.

The postnatal development of the central visual pathways depends on the quality of the visual environment. During the critical period alterations in the conditions of visual input can modify the physiological properties of visual cortical cells. If a monkey is monocularly deprived during the first few weeks of life there is a reorganization of the striate cortex such that the ocular dominance columns representing the functioning eye extend beyond their boundaries and broaden at the expense of the adjacent columns representing the deprived eye. Eventually, most of the striate cortex may be incorporated into a monocular visual system that serves only the deprived eye. The organization of the lateral geniculates (dLGN), the locus of the cell bodies of the terminals in striate cortex, remains normal.

Our specific aims for this fiscal year were:

1. To determine the effects of acute monocular occlusion on local rates of protein synthesis in the laminae of the dLGN in 25 and 50 day old rhesus monkeys. These animals will serve as controls for our chronic monocular deprivation groups. The addition of these 2 groups of animals will make it possible to test for the statistical significance of changes with chronic deprivation.
2. To determine the effects of chronic binocular deprivation of local rates of protein synthesis in 25 day old monkeys. These experiments were designed to examine whether the changes found in local rates of protein synthesis with chronic monocular deprivation are the causes or the consequences of the reorganization which takes place in the striate cortex.
- 3) To examine the effects of our reverse-suture procedure on eye movements. During the course of the experiments in which monkeys were reverse-sutured it was noted that a nystagmus develops during the second 25 days. In collaboration with Dr. R. Tusa, Johns Hopkins Medical School, eye movements are being recorded in these animals.

Methods Employed:

Local rates of cerebral protein synthesis were determined with the [^{14}C]leucine method (Smith et al., 1984).

Unilateral or bilateral tarsorrhaphies were performed under ketamine anesthesia on newborn and 25 day old rhesus monkeys. In some animals a reverse-suture paradigm was used, i.e., unilateral lid suture was performed at birth and at 25 days the sutured eye was opened and the other eye was closed.

Eye movements were recorded with scleral search coils that were implanted at the time of the reverse-suture.

Major Findings:

In our early studies the effects of acute and chronic monocular deprivation in the newborn rhesus monkey on rates of protein synthesis in the laminae of the dLGN were examined. The results showed that in newborn monkeys acute monocular deprivation produced no differential changes in rates of protein synthesis in any of the dLGN laminae. Chronic monocular deprivation (from 0-25 days) resulted in decreases of about 15% in the rates of protein synthesis in the laminae innervated by the deprived eye, whereas in geniculate laminae innervated by the functioning eye rates of protein synthesis were normal as compared with monkeys with binocular vision. In order to test the statistical significance of these effects we have completed another control group of animals, i.e. age-matched monkeys with one eye occluded acutely. The rates of protein synthesis determined in the six laminae of left and right LGN in acutely and chronically monocular occluded monkeys were analyzed by Dr. Pettigrew with a 3 factor analysis of variance with repeated measures. The results show a highly significant ($p < .001$) interaction of group (acute/chronic) and condition (deprived/nondeprived). All other interactions and all main effects were not significant. This analysis shows that chronic monocular occlusion results in a significantly different pattern of protein synthesis in the LGN as compared with acute monocular occlusion. Rates of protein synthesis are decreased in the deprived eye laminae of the LGN and unchanged in the nondeprived eye laminae. These effects on protein synthesis occur when the deprivation is begun at a point in the critical period when there is considerable overlap of the representation from the two eyes in layer IV of striate cortex. These results suggest that the underdevelopment of the deprived columns in striate cortex may be the result of inadequate growth and/or maintenance of axon terminals with consequent default of synaptic connections to the normally maintained terminals of the functional pathway.

In experiments in which monocular deprivation is carried out later in the critical period (from 25-50 days) when there is less overlap in striate cortex, the primary effect is on the nondeprived laminae of the dLGN. In these experiments the nondeprived laminae have increased rates of protein synthesis in comparison to normal, age-matched controls with binocular vision, and the deprived laminae are unchanged. In order to test the statistical significance of these results we have completed an additional control group, i.e. age-matched monkeys with one eye occluded acutely. These experiments have been carried out and are currently being analyzed. The initial results indicate that there is no significant difference in rates of protein synthesis in the laminae of the dLGN between the 50 day old animals with binocular vision and those with acute monocular occlusion. Thus, the reorganization that occurs as a result of the monocular

deprivation from 25-50 days may require a more active process than the default that was seen when monocular deprivation was begun at birth.

In order to determine if the changes in protein synthesis are the causes or the consequences of the reorganization of striate cortex, we have studied the effects of binocular deprivation. Physiological experiments have shown that binocular deprivation during the critical period has no effect on the organization of striate cortex. Rates of protein synthesis were determined in the 6 laminae of the dLGN in 10 25-day old monkeys with normal binocular vision and 8 25-day old binocularly deprived animals. The results were analyzed by Dr. Pettigrew with a 2-factor analysis of variance with repeated measures. The results show no significant interaction between groups and laminae, no significant main effect of group and a significant effect of laminae ($P < .001$), i.e. laminae 2 has a lower rate of protein synthesis than the other 5 laminae. The results of this analysis show that there is no significant effect of chronic binocular deprivation from 0-25 days on the rates of protein synthesis in the dLGN. The results of these experiments were presented at the 1987 meeting of the International Symposium of Cerebral Blood Flow and Metabolism.

During the course of the experiments in which monkeys were reverse-sutured it was noted that a nystagmus develops during the second 25 days. In collaboration with Dr. R. Tusa, Johns Hopkins Medical School, eye movements are being recorded in these animals. In one experiment eye movements were normal on Day 25 at the time of the reverse-suturing. Eye movements became progressively abnormal during the ensuing 25 days and resembled somewhat the nystagmus associated with congenital blindness in humans. A second animal is being studied under these conditions. Eye coils were implanted in both eyes and the progressive development of the nystagmus is being followed. The abnormal movements are conjugate and did not decrease in intensity when both eyes were opened. These studies indicate that this is a good experimental model of congenital nystagmus. A manuscript of these results is in preparation.

Significance to Biomedical Research and the Program of the Institute:

Plasticity, the capacity of the nervous system to respond to changes in the environment, is one of the most fundamental properties of nervous tissue. Learning, a form of plasticity, is a process of intense interest to neuroscientists the world over. In an attempt to study some of the biochemical processes underlying plastic changes, we have embarked on this study of the developing monkey visual system about which the physiology and anatomy are well known. Studies with the [14 C]leucine method for local rates of protein synthesis and the [14 C]deoxyglucose method for local rates of glucose utilization are directed at first a description of some of the biochemical events which occur and then a determination of the regulation of these events. The understanding of these events may provide some insight into the unique properties of the critical period which make it so responsive to environmental manipulation. In addition, this research may have some direct implications on the clinical management of children with congenital cataracts and strabismic amblyopia.

Proposed Course:

The recent experiments on acute monocular occlusion will be analyzed, the statistical analyses will be carried out and manuscripts will be prepared. Experiments on the congenital nystagmus model (in collaboration with Dr. Tusa) will be continued. Control experiments such as binocular deprivation will be carried out. Strabismus produced at birth by surgical section of ocular muscles in conjunction with monocular deprivation will be examined for its ability to produce the abnormal eye movements.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02220-04 LCM

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regional Biochemical Changes in the Normal Aging Brain

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P. I.: Carolyn B. Smith Research Chemist LCM, NIMH

Others: Louis Sokoloff Chief LCM, NIMH

Ernesta Palombo Visiting Fellow LCM, NIMH

COOPERATING UNITS (if any)

Department of Physiology-Anatomy, University of California, Berkeley, CA
(M. C. Diamond)

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies have been carried out on the effects of aging on cerebral protein synthesis and glucose utilization in rats. With the application of local methods developed in this Laboratory, discrete regions of the brain can be examined in normal conscious animals. The regional changes in glucose utilization indicate that entire sensory pathways are affected by the aging process. The fact that similar changes are found in the same pathways with respect to protein synthesis suggests that some of these changes reflect an adaptation of the nervous system to a chronic lack of input. Our findings of age-dependent decreases in glucose utilization in the striatum have been followed up with studies of the effects of aging on the metabolic responsiveness to the dopaminergic agonist apomorphine.

Project Description:Objectives:

The overall purpose of these studies is to examine the effects of normal aging in rats on rates of regional metabolic processes in the brain. In our previous studies, decreases in rates of glucose utilization with age were found in the brain as a whole. On a local level senescent decreases in glucose utilization were found with the most profound effects in the components of the primary auditory and visual pathways. These were effects similar to those seen following acute sensory deprivation of these systems. These results raised the question of whether or not some of the central nervous consequences of normal aging are due to sensory deprivation due to sense-organ degenerative changes, inasmuch as there is known to be some retinal and inner ear degenerative change with age. With only a few exceptions, the rates of glucose utilization in structures of the limbic and motor systems remained unchanged with age. The exceptions were several regions of white matter and the caudate-putamen.

Measurements of energy metabolism do not differentiate between the immediate functional demands of cerebral structures and the longer term maintenance processes within the nervous system. Long term effects that are related to changes in morphology, structural maintenance, and remodeling in the nervous system are more likely reflected in biosynthetic biochemical processes, such as protein synthesis. In another study the effects of aging on local rates of protein synthesis in brain were examined by means of the quantitative autoradiographic [^{14}C]leucine method. The results show that aging is associated with significant decreases in rates of protein synthesis in the brain as a whole, as well as in several specific brain regions. Brain regions involved in visual and auditory function were selectively affected, perhaps due to a chronic lack of sensory input. Several regions involved in motor function and two areas in the limbic system had significantly decreased rates of protein synthesis in the old rats. Notably, there was a significant age-related decrease in protein synthesis in the locus coeruleus which contains the cell bodies of origin of the major ascending noradrenergic innervation of the cortex.

There were two major objectives of the ongoing work during this fiscal year: 1) To study the functional consequences of senescent changes in the nigrostriatal dopaminergic system by determining the effects of normal aging on the metabolic responsiveness of dopamine-receptor activation of systemically-administered apomorphine, and 2) To examine the effects of environmental enrichment in adult rats on resting local rates of cerebral glucose utilization.

Methods Employed:

Studies of the effects of age on the metabolic responsiveness to apomorphine:

In this study Fisher 344 male rats were obtained from the colony maintained by the National Institute on Aging. Three age groups were studied: young adults, 4-6 months of age; middle-aged rats, 14-16 months of age; and old rats, 23-25 months of age. The [^{14}C]deoxyglucose method (Sokoloff et al., 1977) was used to determine local rates of cerebral glucose utilization. Rats were administered with apomorphine at 0.5 mg/kg, 1.5 mg/kg or 5.0 mg/kg or normal saline vehicle

10 minutes before the administration of [^{14}C]deoxyglucose. Behavioral and physiological responses were monitored throughout the study.

Major Findings:

Effects of age on metabolic responsiveness to apomorphine:

The results of our pilot study showed significant dose-dependent effects of apomorphine in 6 of the 14 brain regions examined in the young rats. In the lateral habenula and anterior cingulate cortex the effect of apomorphine was to decrease the rate of glucose utilization, whereas in the subthalamic nucleus, inferior olivary nucleus, substantia nigra (pars compacta), and substantia nigra (pars reticulata), apomorphine stimulated glucose utilization. Age-dependent changes in responsiveness to apomorphine were found in the subthalamic nucleus, substantia nigra (pars reticulata), and inferior olivary nucleus. In the subthalamic nucleus the stimulation of glucose utilization by apomorphine was decreased in the old rats at all doses, including those that elicited maximal responses. In the inferior olivary nucleus and the substantia nigra (pars reticulata), the dose-response curves were markedly depressed in the aged group. No significant effect of apomorphine on the rate of glucose utilization was found in the caudate-putamen as a whole, but a significant stimulation of glucose utilization was found only in the ventral portion in the young animals. The significant age-dependent decreases in responsiveness to apomorphine found in the subthalamic nucleus, substantia nigra (reticulata), and inferior olivary nucleus may reflect the functional consequences of the reported loss of dopamine receptors in the caudate-putamen with aging. Because the results of this pilot study were promising, a complete study has been undertaken.

Studies have been completed on 72 rats. The integrated plasma specific activity of deoxyglucose has been calculated for each of the animals. The autoradiographs of brain sections have been prepared. The resulting 216 films are being analyzed with the aid of our image processing system. Rates of glucose utilization are being determined in 42 brain structures and in the brain, as a whole. When all the results have been tabulated, a dose-response curve for the effects of apomorphine on local rates of cerebral glucose utilization will be constructed for each age group, and each brain structure examined.

Effects of Environmental Enrichment on Local Rates of Cerebral Glucose Utilization:

The work of Renshaw, Diamond and others has shown that environmental enrichment can result in histological changes in the cerebral cortex of adult rats. As environmental enrichment may be the converse of sensory deprivation, we have examined the effects of enrichment on local rates of cerebral glucose utilization. In our studies, results from four "enriched" rats were compared with results from six "standard" rats. Brain regions examined included eight cortical areas, nine limbic areas, ten other subcortical areas, two regions of white matter and the reticular formation. Of the 30 brain regions examined, there were no significant changes in 28 of these. There were no significant increases in any of the regions examined in the "enriched" animals. In both frontal and parietal cortex, rates of glucose utilization were decreased

increases in any of the regions examined in the "enriched" animals. In both frontal and parietal cortex, rates of glucose utilization were decreased (p .05) by 13% in the "enriched" rats. Whether or not these effects are random events, or are actually due to the experimental conditions, can only be determined by a second series of experiments. This work was presented at the November 1986 Annual Meeting of the Society of Neuroscience.

Significance to Biomedical Research and the Program of the Institute:

Insofar as aging is rapidly becoming a problem of increasing social significance, this research, which is focused on senescent changes in the ability of the brain to function, may be of considerable importance to the medical community. Furthermore, our results indicate that some of the changes that occur with age may be the consequence of a decreased functional activity. Confirmation of this possibility, and further understanding of the basic biochemical processes underlying plastic changes in the nervous system of either an involuntal or developmental nature, may be useful in trying to prevent and/or reverse such senescent changes.

Proposed Course:

Completed experiments on the effects of aging on the metabolic responsiveness to apomorphine are currently being analyzed. When the analysis is completed a manuscript of this work will be prepared.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02308-02 LCM

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Growth and Development of Dopaminergic Neurons

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Bernard F. Driscoll Research Biologist LCM, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

NIMH, Bethesda, Maryland 20892

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.5

PROFESSIONAL:

1.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Development of central nervous system pathways involves the action of soluble factors on target cells and interactions between various cell types. One of the best defined CNS pathways is the nigrostriatal pathway. To examine events involved in the formation of this pathway, development of dopaminergic neurons from the embryonic rat mesencephalon was examined in dissociated cell cultures.

Cells were grown in serum-free medium or serum-containing medium in the presence or absence of the basement membrane component, laminin. Mesencephalic cells were obtained from embryonic day 13 to embryonic day 15 rat brains; cells from other regions were obtained from embryonic or postnatal rat brains at times when subsequent in vitro development was shown to be optimal. Development of the dopaminergic neurons in the mesencephalic cultures was determined by uptake of exogenous, labelled dopamine.

Mesencephalic dopaminergic neurons grown alone developed vigorously in culture for 9 days. After this time development ceased. In contrast, if mesencephalic neurons were grown in the presence of cells from other brain regions, the cultures continued to develop to day 20 or day 27. Glial cells from these other brain regions appear to be important for the continued development of the mesencephalic neurons. There also appeared to be a later stage of development or cell survival that was dependent on the presence of specific target cells in the culture. Striatal neurons are the normal in vivo target for most of the mesencephalic dopaminergic neurons. Cells from the striatum provided optimum in vitro conditions for development of the dopaminergic neurons.

Project Description:Objective:

To determine the factors involved in development of central nervous system pathways by observing survival and development of neurons in dissociated cell cultures.

Methods:

Cells were prepared from specific regions of embryonic day (ED) 13 to postnatal day 8 rat brains. Five specific regions were used; the ability of cells from any particular region to grow in vitro is dependent on the time in embryonic development when they are removed. Therefore, cells must be obtained from embryos or neonates of various ages.

The cells were cultured in vitro in either serum containing medium (SCM-10% fetal calf serum) or in serum-free medium (SFM). Use of SFM allows for greater control over factors to which the cells are exposed. On the other hand, cultures in SCM are morphologically different than those in SFM; this provides additional conditions under which to examine the cells' development.

We are interested in assessing the development of the mesencephalic dopaminergic neurons, and in particular the effect of target (striatal) neurons on this development. The degree of development was determined by quantitating the level of dopamine uptake by the reuptake system which is present in all dopaminergic neurons. The uptake of ³H-labelled dopamine was measured by liquid scintillation counting or (for qualitative morphologic studies) by autoradiography. To identify and quantitate the cell types present, representative cultures were fixed, permeabilized and exposed to antibodies which recognize various cell-type specific antigens, particularly antigens present in neurons as astroglia. After exposure to a second antibody containing a fluorochrome, the cultures were examined by fluorescence microscopy.

Major Findings:

One reason for culturing cells in SFM is to control the proliferation of astroglia. In SCM, these cells, if unchecked by mitotic inhibitors, will completely overgrow the neurons. In SFM, the proliferation of astroglia is inhibited presumably due to the lack of growth factors normally present in fetal calf serum. Surprisingly, when the SFM cultures were examined for the presence of astroglia using an antibody to the glial-specific antigen glial fibrillary acidic-protein (GFAP), glia were found to be present in almost all cultures at d-9. More important was the observation that the amount of GFAP was increased in cultures at d-20 and even more so by d-27. We do not know whether the increase in GFAP represents astroglial proliferation or process development. Regardless, while astroglia do not overgrow these cultures, they do represent an unexpectedly large component of the cultures grown in SFM.

Like all neurons in the CNS, the mesencephalic dopaminergic neurons can grow in vitro but to do so they must be removed from brains at a narrow, defined time of embryonic development. Dopaminergic neurons from embryonic day 13 (ED13) brains

grow vigorously in low cell numbers and are exquisitely sensitive to the basement membrane component laminin. ED14 cells grow slightly less vigorously and require higher cell numbers to develop. They have a moderate to good response to laminin. ED15 cells grow poorly unless cultured in high cell numbers and have moderate sensitivity to laminin. By ED16, mesencephalic dopaminergic neurons fail to grow even in the presence of laminin. Other types of neurons in the ED16 brain grow vigorously in culture but these cultures contain no demonstrable dopaminergic neurons.

Mesencephalic dopaminergic neurons examined in our experiments were taken from ED13 to ED15 brains. To assess the effect of target neurons on this development, the dopaminergic neurons were cultured in the presence of neurons from the striatum (ED16-17), the cortex (ED16), the hippocampus (ED19) and the cerebellum (postnatal days 5-8). At the times noted, cells from these regions grew well in vitro in SFM, which was used in most of the experiments.

Mesencephalic dopaminergic neurons developed extremely well when cultured alone. However, some time after d-9 in vitro, these cells show a decline as assessed morphologically and by the dopamine uptake assay. If they are cultured in the presence of cells from other regions, this decline does not occur and in many cases their development increases to d-20 or d-27 in vitro. Dopaminergic neurons cultured with striatal cells display excellent development; however, dopaminergic neurons cultured with cells from other regions also develop well but not as consistently as when striatal cells are in cultures. As mentioned previously, most of these cultures contain glial cells in unexpectedly large amounts. These astroglial cells could be masking any specific effects that target neurons might have on development.

The current working hypothesis is that the mesencephalic dopaminergic neurons can survive and develop when cultured alone for at least 9 days. They must receive all necessary factors from endogenous mesencephalic cells until this time. After day 9 the dopaminergic neurons depend on the presence of cells outside the mesencephalon for continued development. These cells could be glial cells but this has not yet been proven. As has been demonstrated in other studies, it is possible that the dopaminergic neurons produce (specific?) factors that induce the glial cells to in turn secrete factors critical for the development of the dopaminergic neurons. Finally, long term survival could be dependent on the presence of target neurons in culture.

Proposed Course:

Current experiments are designed to define the role of astroglial cells or factors produced by these cells in the survival and development of the dopaminergic neurons in cultures. For instance, since the mesencephalic dopaminergic neurons do not continue to develop when cultured alone, the mesencephalic astroglia (which are present in these cultures along with the neurons) must be qualitatively different than the glia from other brain regions. Also, the simple physical presence of glial cells in culture appears to be insufficient to induce neuronal development. Factors secreted by the glial cells are probably of critical importance.

Another area of interest is the communication between the neurons and the glia that leads to glial enhancement of neuronal development. A deficit in this communication may be the reason that mesencephalic astroglia do not support the development of the mesencephalic neurons: the glia do not respond to the signals produced by the neurons and do not produce the requisite factors. If glial soluble factors can be identified and are sufficient to allow neuronal development, the presence of glial cells can be diminished or eliminated from cultures and the effect of target neurons on the development and terminal survival of dopaminergic neurons can be determined.

Concurrent with the above studies, we have developed techniques for growing predominantly glial or neuronal cultures from various brain regions. These cultures will be used in collaboration with other investigators in the lab to examine CNS energy metabolism with emphasis on the kinetics of glucose metabolism including studies involving control of glucose transport.

Significance to Biomedical Research and the Program of the Institute:

Neurological disorders, whether accompanied by obvious pathologic changes or more subtle, as yet undetected, changes are all due to malfunctioning CNS cells. Knowledge about the mechanisms by which these cells develop into organized pathways and are maintained over time is of obvious importance. Studying the subtle signals involved in brain development in vivo has proven to be difficult due to the compactness, complexity and relative inaccessibility of the brain. Removing the developing cells from this environment and growing them in a more controllable environment in vitro allows ready access to the cells for observation and manipulation. There is no doubt that increased knowledge about the normal development and maintenance of these pathways will provide information useful for the detection of abnormal development or maintenance that leads to mental disorders.

There are limitations to in vitro studies and these observations must always be related to in vivo (or in the case of humans - clinical) observations. A judicious blending of data from these two areas will eventually provide a solution to the puzzle of devastating neurological diseases.

Publications:

Levenbook, I., Elisberg, B., and Driscoll, B.: Rhesus diploid rabies vaccine (adsorbed): neurological safety in guinea pigs and Lewis rats. Vaccine 4: 225-227, 1986.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02307-02 LCM

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Role of Proteinases in Production and Control of Neuropeptides

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.: Marian W. Kies Chemist LCM, NIMH

Others: Gladys E. Deibler Research Chemist LCM, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has temporarily been postponed due to the prolonged absence of the Principal Investigator on account of illness. It is planned that the research will resume the beginning of FY 1988.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 Z01 MH 00507-05 LCM

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Brain Imaging

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Robert M. Cohen, M.D., Ph.D., Chief, CBI, LCM, NIMH

COOPERATING UNITS (if any)

Clinical Neuroscience Branch, NIMH (D. Pickar); Clinical Center, NIH (C. Channing and R. Carson); Child Psychiatry Branch, NIMH (A. Zametkin)

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Clinical Brain Imaging

INSTITUTE AND LOCATION

National Institute of Mental Health
 9000 Rockville Pike, Bethesda, MD 20892

TOTAL MAN-YEARS:

8.5

PROFESSIONAL:

4.5

OTHER:

4.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major areas of effort in this project have been (1) to develop new tracers or other approaches for the study of neurotransmitter function in normal and abnormal physiology; and (2) to apply what tracer methodologies we have available to the study of neuropsychiatric disorders. To these ends, the following achievements are notable. A series of studies of [18F]-cyclofoxy have been completed in baboons that delineate its usefulness as a measure of opiate receptor avidity. An application for its use in humans has been submitted to the Food and Drug Administration. We found that we could successfully apply PET measurement of glucose metabolism to determine biological determinants of attention as we observed for what we believe to be the first time a direct relationship between the metabolic activity of a brain region, the mid-prefrontal cortex and quantitative measures of the accuracy of ongoing performance of auditory discrimination in normals. In patients with schizophrenia, even those who performed as well as normals, the metabolic rate in the middle prefrontal cortex was found to be significantly lower than normal and unrelated to performance. Furthermore, preliminary analysis suggests that medicated patients with schizophrenia demonstrate a similar relationship between the middle prefrontal cortex and performance as normal controls. The findings point to a role of the mid-prefrontal cortex and its dopamine neurotransmitter pathway input in sustained attention and to dysfunction of this region and of its dopamine modulation in some patients with schizophrenia.

OTHER PROFESSIONAL PERSONNEL

Thomas Nordahl, M.D., Ph.D., Medical Staff Fellow, LCM, NIMH
 Michael Gross, M.D., Medical Staff Fellow, LCM, NIMH
 Steven M. Larson, M.D., Chief, NM, CC, NIH
 David Pickar, M.D., NSB, NIMH
 Robert M. Post, M.D., Chief, BPB, NIMH
 Daniel R. Weinberger, M.D., ETB, St. Elizabeths Hospital
 William Semple, Ph.D., Psychologist, LCM, NIMH
 John Cappelletti, Computer Programmer Analyst, LCM, NIMH
 A. Catherine King, Psychology Technician, LCM, NIMH
 Mary S. Dowling-Zimmerman, Psychologist, LCM, NIMH
 Michael Channing, NM, CC
 Dale Kiesewetter, NM, CC
 Alan Zametkin, M.D., CPB, NIMH

MAJOR FINDINGS

Method Development:

In the last annual report, we summarized our early findings with a new selective opiate receptor dependent tracer [18F]cyclofoxy. We have now completed a series of studies demonstrating the hoped for displacement of cyclofoxy binding with the selective opiate receptor antagonist naloxone in baboons. This data is currently being analyzed by two different compartment models to determine a best fit for the data. Furthermore, the quality control issues for the reliable and safe production of cyclofoxy for administration to humans have been settled. An application for the use of cyclofoxy in normals and neuropsychiatric patients has been submitted to the Food and Drug Administration.

Patient Studies with PET:

Early studies by several groups including the intramural NIMH had indicated that either while receiving electric shock or while resting, patients with schizophrenia when compared to normal controls appeared to have relatively lower levels of frontal cortex to posterior cortex brain activity (metabolism), i.e. be hypofrontal. Moreover, the "normally" observed relatively greater metabolism in the frontal cortex, a region somewhat specialized for higher cognitive function, compared to the more posterior cortical regions of the brain that may be more closely associated with primary processing, suggested to investigators that the hyperfrontal pattern of metabolism represented an important dimension of normality, and that the observation of "hypofrontality" in schizophrenia was a reflection of the "executive" function pathophysiology of schizophrenia.

We were concerned, however, by several problems with the approach. First, hypofrontality failed to be observed in some studies. Second, the finding, even in the positive studies, was frequently dependent upon idiosyncratic definitions and selective statistical analysis. Third, affectively disordered patients, even while not substantially depressed, appeared to be more hypofrontal than patients with schizophrenia. Moreover, we felt that the

normal or abnormal functioning of the frontal cortex should in many instances be evaluated in the context of a specific behavioral requirement which is difficult to establish in subjects at rest or who are receiving "meaningless electric shock".

Nevertheless, we attempted to assess the importance of the hypofrontality concept by (1) employing analogous measures of hypofrontality as those used in prior investigations (2) measuring the associations between clinical symptoms and hypofrontality and (3) observing if the chronic administration of neuroleptics, the treatment of choice in schizophrenia, would substantially effect this index. This approach was not fruitful in establishing the significance of the findings of hypofrontality in schizophrenia.

To remedy the above problems, we elected to study frontal cortical function in schizophrenia in the context of a specific executive function, maintenance of directed attention. A continuous performance test (CPT) based on auditory discrimination was chosen because CPTs have consistently been reported to demonstrate deficits in the maintenance of directed attention in schizophrenia and are presumed to be associated more directly to genetic errors than the overt symptomatology of schizophrenia. Furthermore, as the frontal cortex is quite large in man and consists of a number of functionally somewhat independent entities, we elected to do a detailed anatomical examination of this area and the rest of the brain. This meant that we needed to study a large number of normals both in the resting state and while performing CPT so as to allow for a statistical analysis that would reveal replicable results while retaining some degree of power.

We found that we could successfully apply the FDG-PET methodology to determine biological determinants of attention, i.e. those anatomical structures that may contribute to the reception and modulation of sensory stimuli. We observed metabolic rate differences in the middle prefrontal, cingulate and superior posterior parietal cortices of normals performing auditory discrimination compared to resting subjects or subjects receiving electric shocks (Cohen, et al., 1987). Furthermore, a direct relationship was observed between metabolic rates in the middle prefrontal cortex and the accuracy of a normal subject's auditory discrimination. We believe that this is the first time that the metabolic activity of a brain region has been specifically linked to quantitative measures of the accuracy of ongoing performance in normals.

In patients with schizophrenia, even those who performed as well as normals, the metabolic rate in the middle prefrontal cortex was found to be significantly lower than normal and unrelated to performance (Cohen, et al., 1987). Furthermore, we have partially completed our analysis of the data of 8 patients who were receiving neuroleptics and find that the medicated patients with schizophrenia demonstrate a clear association between brain activity in the mid-prefrontal cortex and performance (Cohen, et al., in press). The findings point to a role of the mid-prefrontal cortex and its dopamine neurotransmitter pathway input in sustained attention and to dysfunction of this region and of its dopamine modulation in some patients with schizophrenia.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

The central tenet of the Laboratory is the belief that our increasing knowledge of the molecular genetics, biochemistry and cytology of the central nervous system will fall short of allowing us a complete understanding of normal and abnormal behavior regardless of the degree of sophistication that genetic probes and postmortem analyses achieve without studies of the functioning system. Advances in the understanding of the functioning system are required if we are to delineate the pathophysiology of psychiatric illnesses such as schizophrenia. If for no other reason such work should facilitate a reduction in the heterogeneity of patient samples in research studies of psychiatric disorders by ensuring that patients who have the syndrome of schizophrenia also share the same pathophysiology. This may be of particular importance to genetic studies.

Furthermore, were a single gene found to be principally responsible for the genetic determination of schizophrenia prior to the elucidation of the pathophysiology, schizophrenia researchers would still need information about pathophysiology to tackle the difficult problems of determining the mechanisms responsible for the development of the phenotype and to improve treatment strategies.

At the NIMH-IRP program we have made a sustained effort to use positron emission tomography (PET) to help elucidate the pathophysiology of psychiatric disorders. We have made this commitment because we believe that PET, one of a number of new brain imaging technologies with its unsurpassed ability to precisely localize and quantify in the human brain tracers used for the study of physiological processes, offers the greatest promise for delineating the pathophysiology of schizophrenia.

In our most recent PET studies we chose to examine the functional localization of the ability to perform continuous auditory discrimination because consistent deficits in continuous performance had been reported in schizophrenia and in subjects at increased risk for schizophrenia and because sustained attention is also fundamental to the development and execution of all "goal-directed" behavior. These defects may lie closer to the primary defects presumed to be associated with genetic errors than the overt symptomatology of schizophrenia (fully expressed phenotype). We were able to observe dysfunction of the middle prefrontal with respect to sustained attention in schizophrenia. Although the importance of this dysfunction for understanding the pathophysiology of schizophrenia remains to be delineated, preliminary evidence of the dopamine dependence of this function in schizophrenia as evidenced by change in prefrontal cortex function with neuroleptic treatment suggests that detailed studies of anatomic and neurotransmitter pathways involved in attention are warranted.

PROPOSED COURSE:

The majority of subjects that have participated in our protocols to date were scanned on the ECAT II scanner. The resolution of the ECAT II scanner was 1.8 cm. and the number of slices that could be obtained was at best 7. Furthermore, the attenuation corrections were calculated and could not be

determined empirically, thus limiting the brain structures that could be accurately examined. The Scanditronix scanner now in use for this protocol has a 5 mm. resolution, can gather 28 slices from a single scan, and an empirically derived attenuation correction can be made. Thus, work with the Scanditronix should allow us to replicate our previous findings and search for additional biological determinants of attention and abnormalities in schizophrenia. The use of other populations, e.g., neuropsychiatric disorders, including adult attention deficit disorder (ADD) and the examination of patients while on medications for their disorders (e.g. while ADD patients are on stimulants) should help with the search for additional biological determinants of sustained attention and the delineation of the specificity of these findings for schizophrenia.

Although the success that we have had in pursuing an understanding of the brain metabolic map of sustained attention and a component of its neurotransmitter dependence is gratifying, it is very likely that the study of other cognitive processes will be required to define the pathophysiology of an illness as complex as schizophrenia. Just as no one phenomenological variable such as hallucinations is sufficient to delineate schizophrenia, we will probably need to evaluate brain activity with respect to a number of cognitive tasks as well as with respect to the other major component of behavior, emotion if we are to arrive at an understanding of complex neuropsychiatric disorders such as schizophrenia. Therefore, we will also be pursuing biological determinants of other components of cognition and emotion.

PUBLICATIONS:

Kiesewetter, W.C., Eckelman, W.C., Cohen, R.M., Finn, R.D., and Larson, S.M.: Syntheses and D2 receptor affinities of derivatives of spiperone containing aliphatic halogens. Appl. Radiat. Isotopes 37: 1181-88, 1986.

Ostrowski, N.L., Burke, T.R., Jr., Rice, K.C., Pert, A., and Pert, C.B.: The pattern of ³H-cyclofoxy retention in rat brain after in vivo injection corresponds to the in vitro opiate receptor distribution. Brain Res. 402: 275-286, 1987.

Post, R.M., DeLisi, L.E., Holcomb, H.H., Udhe, T.W., Cohen, R.M., and Buchsbaum, M.S.: Glucose utilization in the temporal cortex of affectively ill patients: Positron emission tomography. Biol. Psychiatry 22: 545-553, 1987.

Cohen, R.M., Semple, W.E., Gross, M., Nordahl, T.E., DeLisi, L.E., Holcomb, H.H., King, A.C., Morihisa, J.M., and Pickar, D.: Dysfunction in a prefrontal substrate of sustained attention in schizophrenia. Life Sci. 40: 2031-2039, 1987.

Cohen, R.M., Semple, W.E., Gross, M., Nordahl, T.E.: From syndrome to illness: Delineating the pathophysiology of schizophrenia with positron emission tomography. Schizophrenia Bull., in press.

Ostrowski, N.L., Hill, C.B., and Pert, A.: Autoradiographic visualization of sex differences in the pattern and density of opiate receptors in hamster hypothalamus. Brain Res., in press, 1987.

Ostrowski, N.L., Pert, C.B., and Pert, A.: Visualization of opiate receptors in vivo. In France Leslie's (Ed.): Receptor Localization: Ligand Autoradiography. New York, Alan Liss, Inc., 1987 in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02296-02 LCM

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

In Vivo Tomographic Imaging of Dopaminergic Systems and their Turnover

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

C. C. Chiueh Pharmacologist LCM NIMH

COOPERATING UNITS (if any)

Nuclear Medicine, CC, NIH (S. Larson); Office of the Director, IRP, NINCDS (I. Kopin); Dept. of Nuclear Medicine, McMaster Univ. Medical Centre, Hamilton, Ontario, Canada (G. Firnau)

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Clinical Brain Imaging

INSTITUTE AND LOCATION

National Institute of Mental Health, 9000 Rockville Pike Bethesda, MD 20892

TOTAL MAN-YEARS:

3.0

PROFESSIONAL:

2.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have established a neurochemical basis for the use of purified [F-18]-labeled L-6-F-dopa as a presynaptic imaging ligand for brain dopaminergic neurons. A potential clinical use of this PET imaging ligand for brain dopamine in determining degrees of brain damage in parkinsonian patients was demonstrated in this study by using the MPTP-induced primate model of parkinsonism. Despite a high background activity due to contamination, we were able to image striatal dopaminergic neurons in living monkeys by using the Scanitronix PET scanner at the NIH Clinical Center. The striatal [F-18]-labeled dopaminergic activity was diminished in the monkeys that received a partially purified [F-18]-labeled 6-F-dopa and indicates a need to purify further this PET imaging ligand prior to the application of this 6-F-dopa/PET procedure in humans. By using animal models of parkinsonism, we have also evaluated a new SPECT imaging ligand for D2 dopamine receptors, [I-123]-labeled IBZM (3-iodobenzylamide derivative). It has been shown that IBZM specifically binds to D2 dopamine receptors in the brain and is displaceable by both agonists and antagonists of the D2 dopamine receptor. The *in vivo* imaging of the D2 dopamine receptors in the caudate nucleus, nucleus accumbens, olfactory tubercle and the kidney were obtained within thirty minutes following the administration of the radioactively labeled IBZM. A denervation induced decrease in dopaminergic fibers and increase in D2 dopamine receptors in the basal ganglia of experimental parkinsonian animals have been imaged by using these pre- and post-synaptic imaging ligands. Thus, this pre-clinical study has demonstrated potential clinical uses of these imaging ligands for studying *in vivo* the dopaminergic activities in patients with neuropsychiatric or Parkinson disorders.

Other Professional Personnel Engaged on the Research Project:

R.M. Cohen	Section Chief	LCM	NIMH
D. Doudet	Visiting Fellow	LCM	NIMH
P. Douillet	Visiting Fellow	LCM	NIMH
W. Singhaniyom	Visiting Fellow	LCM	NIMH
I. Namura	Guest Researcher	LCM	NIMH
T. Bruecke	Guest Researcher	LCM	NIMH
J.J. Chen	Visiting Fellow	LCM	NIMH
H. Miyake	Visiting Fellow	LCM	NIMH
I.J. Kopin	Director	IRP OD	NINCDS
Y.F. Tsai	Visiting Fellow	NI	NINCDS
T. Kondo	Visiting Fellow	NI	NINCDS
R. Finn	Chemist	NM CC	NIH
S.M. Larson	Director	NM CC	NIH
T.S. Chen	Pharmacologist		FDA
J.L. Sun	Pharmacologist	OB	FDA
K.L. Kirk	Section Chief	LC	NIADDK
D. Furlano	Chemist	LC	NIADDK
H.F. Kung	Professor	Dept. of Nuclear Medicine Univ. of Pennsylvania	
G. Firnau	Professor	Dept. of Nuclear Medicine McMaster University Medical Centre Hamilton, Ontario, Canada	

Project Description:

Objectives:

This research project continues the brain imaging project begun several years ago in the Laboratory of Clinical Science (see Chiueh et al., 1984; Z01 MH 02241-01-LCS).

The first goal of this project is to develop an ideal positron emitting pre-synaptic ligand (either carbon-11 or fluorine-18) for *in vivo* imaging of brain dopaminergic systems and to provide an index of the functional turnover rate of dopamine by positron emission tomographic (PET) scanning procedures.

The second goal is to develop and evaluate gamma emitting post-synaptic ligands (iodine-123) for imaging of D2 and D1 dopamine receptors in the brain by single photon emission tomographic (SPECT) procedures.

Such tomographic brain imaging procedures may prove to be useful for determining brain damage in Parkinson's disease, for visualizing regeneration of striatal dopamine and for evaluation of up and down regulation of dopaminergic activities and receptors in neuropsychiatric disorders.

Methods Employed:

A. Synthesis of Fluorine-18 labeled L-6-fluoro-dihydroxyphenylalanine and iodine-123 labeled 3-iodobenzylamine:

Fluorine-18 labeled L-6-F-dopa is synthesized by Dr. R. Finn using the procedure of Firnau et al. (1984) and Adams et al. (1986). The L-6-F-dopa is separated from 5-F- and/or 2-F-species by using HPLC procedures. [F-18] isotope (2 hr. half-life) is being generated and produced by using the newly installed cyclotron in the NIH Nuclear Medicine Department. Carbon-14 labeled 6-F-dopa is enzymatically synthesized by Furlano and Kirk (1987).

Iodine-123 or iodine-125 labeled 3-iodobenzylamine (specific activity: greater than 1000 Ci/mmol) is produced and purified by Kung et al. (1986).

B. Animals:

Adult rhesus monkeys (*Macaca mulatta*) and/or baboons of both sexes (5-8 kg) were used. These animals were housed individually in primate quarters on a 12-hr light/dark cycle. Purina monkey chow, water, juice, and fresh fruit were given *ad lib*. L-dopa therapy (Sinemet 100/10, q.i.d.) was given to severely affected parkinsonian monkeys. Full and hemiparkinsonism is induced in monkeys after an intravenous or intra-arterial administration of a dopamine neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), respectively.

During the PET procedure, animals were anesthetized with pentobarbital sodium (35 mg/kg, i.v.). A head mold was developed and designed to hold the

head steady in the PET scanner. Acute arterial and venous catheters were placed to monitor blood pressure, for blood sampling, and for injection of tracers.

Rats (200 g) or mice (25 g) were pretreated with peripheral decarboxylase inhibitor (MK-486, 25 to 75 mg/kg i.p., 30-60 min.). Carbon-14 labeled L-Dopa (sp. act. 10.9 mCi/mmol) was administered through a venous catheter placed in the tail vein. Animals were sacrificed at various times after the treatment for autoradiographic procedures and/or neurochemical assays.

C. Neurochemical Procedures:

The metabolites of 6-[F-18]-dopa in plasma and/or brain samples, i.e., 6-F-dopamine, 6-F-dihydroxyphenylacetic acid, 6-F-homovanillic acid and O-methylated 6-F-dopa were separated and quantified by HPLC procedures. A semi-preparatory HPLC system was used for an isolation of radioactively labeled 6-F-dopa.

D. PET Imaging of Brain Dopamine Neurons:

The brain dopamine imaging procedure described by Garnett *et al.* (1983) was used. Briefly, following an intravenous injection of 1.5 to 2 mCi purified L-6-[F-18]-dopa, anesthetized monkeys were examined in a 10 mm horizontal brain slice along the orbitomeatal plane in the Scanditronix PET scanner for 2 to 4 hours. At various intervals, blood samples were drawn and assayed for 3-O-methyl-6-F-dopa, the major metabolite of 6-F-dopa. Some animals were sacrificed at the end of the experiment. Disc-shaped punches of tissue from the caudate nucleus and the putamen were assayed for dopamine and its biosynthetic enzymes in order to correlate the brain imaging data with the endogenous dopaminergic activities.

E. Autoradiographic Imaging of Brain Dopamine and its Receptors in Small Experimental Animals:

Some of the experimental designs of future clinical brain imaging procedures are being conducted in small experimental animals following an intravenous administration of L-3-[C-14]-dopa, L-2-[C-14]-6-F-dopa and [I-125]=IBZM. Mice or unilaterally-lesioned rats were pretreated with antipsychotic agents (haloperidol), MAO inhibitor (deprenyl), dopamine uptake blocker (amfonelic acid), L-dopa decarboxylase inhibitor (NSD-1015), and major tranquilizer (reserpine) in order to manipulate dopaminergic activities in the brain. Animals were sacrificed at various intervals after the administration of imaging ligand in order to simulate clinical brain imaging procedures for investigating the turnover rate of dopaminergic neurons. The brain was quickly dissected and frozen in -20 C isopentane. Brains were cut frozen into 30 um thick sections which were mounted on gel-coated slides. Sets of serial sections through the striatum, hypothalamus and mid-brain were processed for autoradiographic demonstration of brain dopaminergic systems using LKB ultrafilm. The autoradiographic imaging was quantified by using a computerized densitometer and external radioactivity standards.

F. Imaging of D2 Dopamine Receptors by SPECT Procedures:

Radioactive iodo-amphetamine has been used in SPECT procedures for *in vivo* imaging of blood flow of the human brain. This clinical procedure is modified for the current preclinical study using subhuman primates.

Significance to Biomedical Research:

The MPTP-induced primate model of parkinsonism was employed in the present preclinical study to evaluate these newly developed PET and/or SPECT imaging tracers for assessment of brain dopaminergic functions in the living organism. The current preclinical results showed clearly that [F-18]-6-F-dopa and [I-123]-IBZM are excellent dopamine imaging ligands and have potential uses as a diagnostic tool in the clinic for not only identifying parkinsonian patients in the subclinical stage, but also for elucidating dopaminergic mechanisms of neuropsychiatric disorders. Further development could lead to the procedure's use in the investigation of dopamine turnover rate and D2 dopamine receptor density in studying neuropsychiatric disease.

Proposed Course:

A. Preclinical Studies:

The 6-F-dopa/PET procedures including the generation of [F-18] gas by a cyclotron, the fluorination of L-dopa, and a partial purification of [F-18]-labeled 6F-dopa, and scanning of living monkeys have been successfully tried at the NIH medical center in living monkeys. Before we apply the brain imaging procedure to human subjects, vigorous HPLC purification and additional toxicological and pharmacological evaluations have to be performed in order to meet the safety guidelines stipulated by the Food and Drug Administration. So far, the rate limiting step of this project is the isolation and purification of [F-18]-labeled L-6-F-dopa.

1. Visualization of Degree of Damage to Brain Dopamine in MPTP-induced Hemi-parkinsonism by the 6-F-dopa/PET Procedures.

The MPTP-induced primate model of parkinsonism is being used extensively for research in dopaminergic functions. These parkinsonian monkeys require L-dopa therapy and sometimes tube-feeding in order to sustain their life. Recently, a hemi-parkinsonian monkey model has been developed. A post-mortem neurochemical assay revealed that the damage to brain dopamine of these monkeys was limited to the infusion side, contralateral to the side of motor dysfunction. Since these monkeys suffered parkinsonism only in one side of their body, the animals still were able to care and feed themselves. Furthermore, each animal can serve as its own paired control in testing these brain imaging ligands designed for PET and SPECT studies.

Triplicate PET scans will be performed on these hemi-parkinsonian monkeys in order to obtain quantitative and statistical information on the performance of the Scanditronix PET scanner. The *in vivo* PET data will be verified at the

end of the experiment by performing ex vivo neurochemical measurement of dopamine, its synthetic enzymes, and metabolites in each animal and histological confirmation of cell death or survival. The correlation between the in vivo PET activity and the degree of parkinsonism may provide standards for future clinical diagnosis of parkinsonian patients even in the subclinical stage.

Major Findings:

This research project on the use of L-6-F-dopa for PET imaging of brain dopamine has been conducted at NIMH since 1981. In collaboration with Dr. Irwin Kopin (NINCDS), Dr. Kenneth Kirk (NIADDK), Dr. C. Robert Creveling (NIADDK), Dr. Ronald Finn (NIH, Nuclear Medicine), and Dr. Gunter Firnau (McMaster University Medical Centre), we have established a neurochemical basis for the use of purified L-6-F-dopa as a presynaptic imaging ligand for brain dopamine and demonstrated potential clinical applications of the 6-F-dopa/PET imaging procedure in collaboration with the McMaster and the NIH nuclear medicine PET imaging groups.

The MPTP-induced brain damage to the basal ganglia in three parkinsonian monkeys was visualized by using purified fluorine-18 labeled L-6-F-dopa and the McMaster PET scanner (Chiueh *et al.*, 1986). This PET procedure is being adapted and tested at the Department of Nuclear Medicine of the NIH Clinical Center. In the control monkeys, the fluorine-18 activity in the striatum of the brain increased by three-fold over the background activity seen in the non-dopaminergic brain regions following the administration of a partially purified 6-F-dopa (specific activity: 100-150 mCi/mmol; 70 to 80 chemical purity; prepared by Dr. R. Finn). The major contaminant of the 6-F-dopa was identified by our quality control procedure as 2-F-dopa which was not decarboxylated to form 6-F-dopamine but was rather O-methylated to 3-O-methoxy-2-F-dopa and thus interfered with the 6-F-dopa imaging of brain dopamine neurons as indicated from the results of our autoradiographic studies in small experimental animals. This partially purified [F-18]-labeled 6-F-dopa was not able to image in vivo brain transplants in parkinsonian monkeys. These results will be reported at the Xth International Congress of Pharmacology (August 23-28, 1987 Sydney, Australia).

It is currently believed that MPTP exerts its toxic effects through its metabolite MPP⁺. We reported that intranigral injection of MPP⁺ caused a dose-dependent depletion of striatal dopamine in rats. In the present study, we used radioactive pre- and post-synaptic ligands in addition to [Ca-45] to visualize autoradiographically MPP⁺ effects on dopaminergic systems. Autoradiographic procedures were performed two weeks after unilateral lesioning of the median forebrain bundle following intravenous administration of [C-14]-L-dopa or [I-123]-IBZM. In controls, the in vivo presynaptic [C-14]-L-dopa imaging revealed dopamine-rich areas, such as the caudate nucleus, the nucleus accumbens, and the median eminence. Dopamine fiber imaging completely disappeared in the striatum of the unilaterally nigrallesioned rats. A denervation-induced dopamine receptor supersensitivity was visualized by using the in vivo [I-125]-IBZM binding and ex vivo

autoradiographic procedures (in collaboration with Dr. Kung of the University of Pennsylvania). D2 dopamine receptors as seen by the [I-125]-IBZM binding increased by 50% in the denervated side of basal ganglia of unilateral-lesioned rats and in hemi-parkinsonian monkeys. The [I-125]-IBZM binding to brain receptors was found to be highly specific to D2 dopamine receptor sites and was displaced by D2 agonists and antagonists. Furthermore, the in vivo imaging of D2 dopamine receptors in the brain were obtained within thirty minutes following the administration of [I-125]-IBZM and also yielded a four to one ratio of specific to nonspecific activity. Our report to the VIIth Catecholamine Symposium (June 14-19, 1987, Jerusalem, Israel) indicates that IBZM is a new SPECT imaging ligand of D2 dopamine receptor when labeled with the short half-life isotope I-123.

2. Turnover of Brain Dopaminergic Systems Following Administration of Antidepressants, Antipsychotics, Tranquilizers, and Antiparkinsonian Drugs:

Efforts will be focused on the PET procedure for measuring the in vivo turnover rate of dopamine in the mesolimbic and mesocortical systems. The previously published procedures of measuring turnover rate of brain dopamine required at least 5-6 animals per group at 3 different time points. The present PET procedure by using a purified [F-18]-6-F-dopa (98% purity) can offer a complete time curve in each animal in vivo following neuropharmacological manipulations.

- a. Dopamine receptor antagonists: Antipsychotics
- b. Dopamine uptake blockers and dopamine releasing agents: amphetamine and cocaine
- c. Electrical activation of the dopaminergic systems: e.g., electrical convulsion shock treatment

3. In vivo SPECT Imaging of D2 Dopamine Receptors by [I-123]-IBZM:

The newly developed D2 dopamine receptor imaging ligand will be tested in a clinical SPECT imaging camera by using a hemi-parkinsonian monkey model. Our in vitro autoradiographic study has demonstrated a 25-50% increase in the binding sites of D2 dopamine receptors in the caudate nucleus and putamen of the MPTP-lesioned parkinsonian monkeys. This SPECT procedure will use [I-123]-labeled IBZM with a 13-hour half-life. This SPECT imaging ligand will be synthesized and provided by Dr. H. Kung of the University of Pennsylvania in this preclinical trial.

B. Clinical Studies:

After passing the preclinical toxicological evaluations, these pre- and post-synaptic dopaminergic ligands will be administered to normal volunteers, parkinsonian patients, bipolar manic-depressive patients, schizophrenic patients, and other patients suffering from disorders which may involve brain dopamine. These clinical projects will be conducted following established NIH guidelines and with the collaboration of different clinical branches of NIH and NIMH.

Publications:

Namura, I., Douillet, P., Sun, C.J., Cohen R.M and Chiueh, C.C.: MPP⁺ (1-methyl-4-phenylpyridine) is a neurotoxin to dopamine-, norepinephrine-, and serotonin-containing neurons. Eur. J. Pharmacol. 136: 31-37, 1987.

Chiueh, C.C.: Dopamine in the Extrapyramidal Motor Function: A Study Based upon the MPTP-induced Primate Model of Parkinsonism. In Joseph, J. (Ed.): Central Determinants of Aged-related Declines in Motor Function. New York, Annals New York Academy of Science, 1987, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00931-14 LGCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characteristics and Regulation of S-Adenosylhomocystein Hydrolase

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	P. S. Backlund, Jr.	Senior Staff Fellow	LGCB NIMH
	R. R. Aksamit	Research Chemist	LGCB NIMH
	G. L. Cantoni	Chief, Laboratory of General and Comparative Biochemistry	LGCB NIMH

COOPERATING UNITS (if any)

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Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

2

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

S-adenosylhomocysteine hydrolase plays a critical role in regulating AdoMet-dependent methylations in eukaryotic cells by regulating the ratio of AdoMet/AdoHcy. Several approaches are being used to determine the structure and function of this enzyme.

1) Structure Determination: The enzyme has been purified from rat liver, and cloned from a rat liver cDNA library. The amino acid sequence was determined and a putative NAD binding site identified. The rat liver enzyme has been expressed in E. coli and site-directed mutagenesis is in progress to determine the function of specific amino acid residues. Conformational changes for active and inactive forms of the enzyme have been examined by fluorescence, and circular dichroism.

2) Ligand Binding and Kinetic Properties: The role of NAD, nucleotide, and cAMP binding in regulating the catalytic activity has been studied, and photoaffinity ligands are being used to label the binding sites. A large number of adenosine and adenosylhomocysteine analogs have been examined for their ability to function as inhibitors and/or substrates of the enzyme.

3) Biological Effects of Inhibitors: In vivo these adenosine analogs can form very potent and specific inhibitors of transmethylation reactions, and these inhibitors have a wide range of biological activities, including antiviral activity against several RNA and DNA viruses, inhibition of leukocyte chemotaxis, and stimulation of cell differentiation.

Other investigators:

J. Kasir	Visiting Fellow	LGCB NIMH
T. Gomi	Visiting Fellow	LGCB NIMH
T. Caryk	Chemist	LGCB NIMH
M. Fujioka	Toyama Medical & Pharmaceutical University, Toyama, Japan	

Project Description:

As is well known, S-adenosylmethionine (AdoMet) is a key intermediate in biological transmethylation and transalkylation reactions. There are hundreds of reactions, each catalyzed by a specific enzyme, that utilize AdoMet as a substrate. It is obvious that the utilization of AdoMet in biological systems must be under regulatory controls, but at the present time little is known about the nature of these controls. It has been established that S-adenosylhomocysteine (AdoHcy), one of the products of transmethylation reactions that utilize AdoMet as methyl donor, is a competitive inhibitor of most reactions in which AdoMet participates. From the result of work in this and other laboratories, it has been proposed that the intracellular ratio of AdoMet/AdoHcy must be of key importance in the regulation of biological alkylation reactions, and that this ratio plays a role in determining the hierarchy of biological methylation reactions. In eukaryotes, AdoHcy is metabolized through a single metabolic pathway by S-adenosylhomocysteine hydrolase (AdoHcyase), an enzyme which catalyzes the reversible hydrolysis of AdoHcy to adenosine and homocysteine. Because of the central role of AdoHcyase in the metabolism of AdoHcy and in maintaining the ratio of AdoMet/AdoHcy, this enzyme has been under intensive study in this and other laboratories.

S-Adenosylhomocysteine hydrolase has been purified from a variety of sources. Previous work has shown that the mammalian enzyme consists of structurally identical subunits, contains four mols of tightly bound NAD/mol of enzyme, and also binds cAMP and adenosine. The chemistry of the catalytic reaction is fairly well understood, but very little is known about the structure of the enzyme and its relation to function. Our studies are directed towards 1) the elucidation of the primary structure of the hydrolase by molecular cloning of its cDNA and by inference, its secondary and tertiary structure, 2) the determination of the specific polypeptide sequences that are involved in its binding, catalytic, and regulatory sites, 3) characterization of the conformational changes that accompany activation and binding of substrates and cofactors, and 4) crystallization of the enzyme to provide an absolute three-dimensional structure by X-ray diffraction.

The enzyme was purified to homogeneity, and the amino acid sequence of several cyanogen bromide and tryptic peptides were determined. The purified enzyme was also used to produce antibodies against the hydrolase, and the antibodies were used to screen a λ gt11 cDNA library from rat liver to clone and sequence the rat liver enzyme. The cloned sequence was used to determine the amino acid sequence of the protein, and a putative NAD binding region was identified by the homology of the amino acid sequence with other NAD binding proteins. The cloned hydrolase was also expressed in *E. coli*, at an induced level reaching approximately 10% of the bacterial proteins. Site directed mutagenesis

is also being attempted, in order to examine structure/function relationships for different regions of the enzyme, such as the NAD and adenosine binding sites. The cloned cDNA sequence is also being used to examine the level of mRNA expression in different cell types, and in other species. In addition, the genomic organization of the hydrolase is also being examined.

The hydrolase has binding affinities for nucleosides and we are investigating the possible role of ATP, adenosine, cAMP, and the tightly bound NAD's in the regulation of the enzyme. We have shown that the enzyme is inactivated by Mg^{++} , ATP, and KCl with the loss of four molecules of NAD, and it can be reactivated upon incubation of the enzyme with NAD. When NAD is bound to the enzyme, little cAMP binds, while more cAMP binds to the enzyme lacking NAD suggesting that the cAMP may bind to the NAD site. Fluorescence, circular dichroism, differential ultraviolet spectroscopy studies, and photoaffinity labeling have been used to monitor changes in conformation of the enzyme upon inactivation and reactivation. The emission and excitation spectra of inactivated enzyme, for example showed a loss in tryptophan fluorescence intensity which appears to be restored upon reactivation. ATP, adenosine, and cAMP have binding affinities for the enzyme and it is not clear how they fit in catalysis and/or regulation. Various affinity reagents have been used to label the hydrolase, and the labeled fragments will be isolated and sequenced to determine the amino acid residues that comprise the active site and/or binding clefts in the protein. The amino acid sequence determined from the cloned cDNA sequence will help to determine the regions of the protein modified by these affinity reagents. Modification of the amino acids at these sites by site directed mutagenesis, will provide independent data on the role of these amino acid residues in catalysis.

While the biochemical mechanisms of transmethylation reactions have been elucidated many years ago, largely as a result of the studies by Cantoni and his collaborators at NIH, the correlation between many methylation reactions and cellular functions remains obscure. For instance, the significance of the methylation of a variety of informational macromolecules, such as proteins and nucleic acids (DNA, ribosomal-, messenger-, viral and tRNA, etc.), or of complex polysaccharides, or even simpler compounds such as guanido acetic acid, nicotinamide, etc., is not immediately obvious and is the subject of much debate. A role for DNA methylation in gene expression has been suggested by observations from several laboratories. We have shown that both 3-deaza-Ado and 3-deaza-Ari can stimulate cell differentiation in a number of cell lines, including the differentiation of myoblasts to form myotubes, and the differentiation of myeloid cells to synthesize globin. It is possible that 3-deaza-Ado may cause differentiation of these cells by inhibiting DNA methylation. However, since 3-deaza-Ado also inhibits a number of other methylation reactions, further work will be required to identify the reactions involved. In collaboration with Dr. Razin, we have proposed a novel mechanism for the transient demethylation of DNA during differentiation where 5-methylcytosine is replaced enzymatically by cytosine, by a mechanism distinct from conventional excision-repair (see Z01 MH 02321-2 LGCB).

Since AdoHcyase is the only enzyme known to metabolize AdoHcy in eukaryotes, inhibition of this enzyme by analogs can be used to alter the ratio of AdoMet/AdoHcy in the cell. We decided some years ago to take advantage of this fact

and initiated a long range experimental project designed to study in depth the properties of AdoHcyase, and then to develop a series of specific inhibitors of this enzyme. As a result of these studies on the properties of AdoHcyase, we have established that the use of specific inhibitors makes it possible to alter the intracellular levels of AdoHcy and/or to accumulate intracellularly congeners of AdoHcy of the general formula S-purinylyhomocysteine (PurHcy). By using these inhibitors, it is possible to modulate the AdoMet/AdoHcy and/or AdoMet/PurHcy ratio in different cellular systems, and to examine the consequences of these changes on cellular functions.

Our studies, confirmed and extended in other laboratories, have identified several inhibitors of AdoHcyase. These compounds have a variety of biological effects and may have important clinical applications, and explain some of the mechanisms of action of some clinically important compounds. Irreversible inhibitors of AdoHcyase include the compounds 9- β -D-arabinofuranosyladenine (Ara-A), 3-deaza-9- β -Darabinofuranosyladenine (3-deaza-Ara-A), and 2-chloro-adenosine. Ara-A has been used by others in chemotherapy for cancer patients. 3-Deaza-Ara-A and 2-chloroadenosine might be expected to have clinical effects similar to Ara-A, since they produce similar inhibition of AdoHcyase. Of the many reversible inhibitors tested, two compounds have been extensively studied in this laboratory as prototype compounds of this group; 3-deazaadenosine (3-deaza-Ado) and 3-deazaaristeromycin (3-deaza-Ari). 3-Deaza-Ado is a potent competitive inhibitor of AdoHcyase with K_i of 5-8 μ M, and as a substrate has a K_m value about equivalent to the natural substrate, adenosine. In contrast to 3-deaza-Ado, 3-deaza-Ari is not a substrate for AdoHcyase, but it is a very potent competitive inhibitor, with K_i of 2.0 nM for the hamster liver enzyme. Neither compound is a substrate for either adenosine kinase or adenosine deaminase.

The capacity of AdoHcyase to synthesize AdoHcy analogs in vivo, as has been shown with 3-deaza-Ado, demonstrates the exciting possibility of synthesizing potent and specific methylation inhibitors intracellularly. Comparison of the biological effects of 3-deaza-Ado and 3-deaza-Ari has made it possible to attribute some of the differences in specificity to the finding that 3-deaza-AdoHcy is a more potent and specific inhibitor of some transmethylation reactions than AdoHcy. We have found that macrophage chemotaxis is specifically inhibited by the intracellular formation of 3-deaza-AdoHcy, brought about by treatment of the cells with 3-deaza-Ado, while chemotaxis is unaffected by accumulation of AdoHcy by treatment with 3-deaza-Ari (see Z01 MH 00942-06). We have further shown that inhibition of chemotaxis by 3-deaza-Ado is correlated with inhibition of the synthesis of specific proteins which are not inhibited by 3-deaza-Ari. The inhibition of synthesis of specific proteins was attributed to an inhibition of mRNA synthesis. Both 3-deaza-Ado and 3-deaza-Ari were used to inhibit mRNA methylation, and the methylation of adenosine on the N-6 position was very sensitive to inhibition, while methylation of the guanosine in the mRNA cap was only slightly inhibited. In contrast, mRNA synthesis was greatly inhibited with 3-deaza-Ado and only partially inhibited with 3-deaza-Ari. Both 3-deaza-Ado and 3-deaza-Ari also inhibit the replication of various RNA and DNA viruses. The sensitivity of various viruses to these two drugs is different, and it seems probable the some of the antiviral effects can be attributed to an inhibition of viral mRNA synthesis or methylation. The specific reaction(s) involved in inhibition of RNA synthesis has not been identified, and the effect of both

compounds on viral RNA methylation may be useful for examining the role of these reactions in the synthesis and processing of different classes of viral RNA.

We have shown that the cytostatic effect of 3-deaza-Ari for RAW264 cells, can be reversed by micromolar concentrations of homocysteine. Since AdoHcy is the only cellular source of homocysteine, we concluded that cells incubated with 3-deaza-Ari cannot recycle methyltetrahydrofolate and regenerate tetrahydrofolate for use in de novo synthesis of purines and pyrimidines. This condition is similar to the situation with vitamin B₁₂ deficiency, which inactivates methionine synthase, and causes methyltetrahydrofolate to accumulate. In addition, it would be expected that cells incubated with 3-deaza-Ari would contain less cystathionine, an amino acid without a known function that is found in high concentration in the brain. These findings could have clinical significance in situations where AdoHcyase is inhibited such as the administration of Ara-A and patients with adenosine deaminase deficiency.

In a series of recent studies in Europe and in this country, it has been found that AdoMet, given parenterally to depressed patients produced rapid and remarkable improvement in the clinical picture. These studies indicate that AdoMet has approximately the same antidepressant activity as the standard tricyclics, such as imipramine, amitryptiline, etc. It is noteworthy, however, that administration of AdoMet is not accompanied by any toxic side effects, and thus, this mode of therapy may represent a considerable improvement over the therapeutic regimens currently in use. The mechanism of action of AdoMet in depressive illness is unknown. It should be pointed out, however, that the dose of AdoMet found to be effective in the management of clinical depression (200-400 mg/i.v./day) is very small compared to the daily flow of methionine through AdoMet. Human adults synthesize and metabolize about 20 millimoles of AdoMet/day, or 20-40 times the dose used in clinical trials.

Significance to Biomedical Research and the Program of the Institute:

Studies of the AdoHcyase and its inhibitors are important to understanding the regulation and function of biochemical transmethylation, and have possible clinical applications in the development of specific inhibitors for certain methylation reactions. Since AdoMet dependent methylation reactions are involved in the synthesis of so many compounds, including DNA, RNA, proteins, lipids, and neurotransmitters, the regulation of these reactions can alter many cell functions. Inhibitors of methylation reactions have been shown to affect cell differentiation, leukocyte chemotaxis, and virus replication. The possible clinical applications could be in the development of compounds for use in chemotherapy, immunosuppression, and antiviral drugs. Because of the important role of methylation in neurotransmitter synthesis, these compounds could have important effects on brain function as well.

Proposed Course of Research:

The cloned cDNA sequence will be used for site directed mutagenesis in order to characterize the role of specific amino acids in the enzyme catalysis. The genomic organization of the hydrolase gene is also being investigated. Studies on several inhibitors will continue in order to determine specific mechanisms of inhibition, and to determine correlations between inhibition of

specific reactions and the physiological effects of these compounds. Much of the work will focus on methylation reactions involved in leukocyte chemotaxis, and on the role of DNA methylation in gene expression. The mechanism for how the pattern of DNA methylation is changed during differentiation will continue to be examined. In addition, the role of different RNA methylations on RNA metabolism and protein synthesis will also be examined.

Publications:

de la Haba, G., Agostini, S., Bozzi, A., Merta, A., Unson, C. and Cantoni, G.L.: S-Adenosylhomocysteinase: Mechanism of reversible and irreversible inactivation by ATP, cAMP and 2'-deoxyadenosine. Biochemistry, 16 8337-8342, 1986.

Backlund, P.S. Jr., Carotti, D. and Cantoni, G.L.: Effects of the S-adenosylhomocysteine hydrolase inhibitors 3-deazaadenosine and 3-deazaaristeromycin on RNA methylation and synthesis. Eur. J. Biochem. 160: 245-251, 1986.

Ogawa, H., Gomi, T., Mueckler, M.M., Fujioka, M., Backlund, P.S. Jr., Aksamit, R.R., Unson, C.G., and Cantoni, G.L.: Amino acid sequence of S-adenosyl-L-homocysteine hydrolase from rat liver as derived from the cDNA sequence. Proc. Natl. Acad. Sci. USA. 84: 719-723, 1987.

Gahl, W.A., Finkelstein, J.D., Mullen, K.D., Bernardini, I., Martin, J.J., Backlund, P., Ishak, K.G., Hoofnagle, J.H., and Mudd, S.H.: Hepatic methionine adenosyltransferase deficiency in a 31-year-old man. Am. J. Hum. Genet. 40: 39-49, 1987.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00936-23 LGCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Homocystinuria: Methionine Metabolism in Mammals

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

S.H. Mudd Chief, Section on Alkaloid Biosynthesis

LGCB NIMH

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0.1

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CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of a 31-year-old man with proven partial deficiency of hepatic methionine adenosyltransferase (MAT) activity have been continued. Identified abnormal metabolites in his bodily fluids, urine, and/or breath include: L-methionine-d-sulfoxide, 4-methylthio-2-oxo-butyrate, 3-methylthiopropionate, dimethylsulfide, and a mixed disulfide $\text{CH}_3\text{S-SX}$, the structure of which is still under investigation. Balance studies have permitted calculation of the fluxes of methyl- and sulfur-containing compounds and shown that: In spite of the deficient activity of hepatic MAT, the patient forms a normal amount of the product of this enzyme, S-adenosylmethionine (SAM). In spite of the normal rate of formation of SAM, the patient does not convert methionine sulfur to sulfate at a normal rate. In spite of his high body load of methionine, the patient conserves methionine by N^2 -methyltetrahydrofolate-dependent methylation of homocysteine. The later observations are explained in terms of the regulatory effects of SAM on cystathionine synthase and methylénetetrahydrofolate reductase. In the presence of 20- to 50-fold elevations of methionine, the transamination pathway metabolizes about 20% of the normal methionine intake of this patient, although the transamination pathway is clearly not sufficiently active to prevent the accumulation of methionine.

Studies of a 31-year-old man with documented partial (93% decrease) deficiency of hepatic methionine adenosyltransferase (MAT) have been continued to answer the questions posed in last year's annual report. MAT activity has been assayed in erythrocytes and cultured skin fibroblasts and found to be normal. The implication is that extra-hepatic form(s) of MAT are under genetic regulation different from that governing the chief hepatic form. Lean body mass has been measured by determination of total body potassium in a whole body counting chamber, and found to be 63.8 kg, or 87% of total body weight. In normal persons this would be reflected by excretion of 14.7 mmol creatinine/day. The patient excreted 13.9 +/- 0.4, very close to the predicted value. His liver is normal on histological examination and by a variety of function tests.

The following sulfur-containing compounds have now been identified in urine, breath, and/or bodily fluids of the patient in abnormal amounts:

- (a) L-Methionine-d-sulfoxide. The presence of one diastereoisomer, only, implies the existence of a here-to-fore undescribed enzyme in humans forming this sulfoxide.
- (b) 4-Methylthio-2-oxobutyrate, the immediate product of methionine transamination.
- (c) 3-Methylthiopropionate, formed by oxidative decarboxylation of the above keto acid.
- (d) Dimethylsulfide, present in 17-fold normal concentrations in the breath of the patient, and accounting for the peculiar breath odor which was his chief complaint.
- (e) A mixed disulfide, $\text{CH}_3\text{-SX}$, which is a quantitatively important urinary excretion product at 2.1 mmol/day. The structure of this hitherto undescribed compound is under investigation.

The patient was placed upon several diets, each with a different methionine intake. Excretions of methyl- and sulfur-containing compounds were measured, both in a steady-state during intake of a diet normal for this individual, and during methionine restriction and supplementation. The results obtained enabled us to construct methyl and sulfur balances, and to arrive at several important and novel conclusions:

- (a) In spite of his proven deficiency in hepatic MAT activity, the patient forms the product of this reaction, S-adenosylmethionine (SAM), at a virtually normal rate of 18 mmol/day. Presumably this is achieved by virtue of the build-up of methionine proximal to the block driving flux through any residual activity of hepatic high K_m MAT. The normal extrahepatic MAT also must contribute, but the extent of this contribution is uncertain.
- (b) In spite of the normal rate at which SAM is formed, the patient does not convert methionine sulfur to sulfate at a normal rate on his usual diet, and does not respond normally to changes in methionine intake with commensurate changes in sulfate output.
- (c) In spite of his extremely high body-load of methionine, the patient carries out a high rate of N^5 -methyltetrahydrofolate-dependent methylation of homocysteine (8.8 mmol/day). He thus reconverts homocysteine to methionine and conserves the latter amino acid under circumstances in which a normal human would not do so.

(d) The apparently anomalous conclusions outlined in (c) and (d), above, can be reconciled and explained in terms of a lack of the regulatory effects of SAM upon cystathionine synthase and methylenetetrahydrofolate reductase. These effects, until now demonstrated only in vitro, each tend to enhance the proportion of available homocysteine which, under conditions of methionine excess, is converted to cystathionine for eventual degradation rather than being conserved by methylation to reform methionine.

(e) The transamination pathway metabolizes at least 20% of the methionine degraded by this patient. This is achieved in the presence of 20-50 fold elevations of methionine above normal. The transamination pathway is clearly not active enough to prevent such accumulations.

Publications:

1. Mudd, S.H.: Homocystinuria, In J.B. Wyngaarden and L.H. Smith (Editors), Cecil Textbook of Medicine, 18th edition, W.B. Saunders, Philadelphia, 1987, in press
2. Mudd, S.H., Levy, H.L., Skovby, F.: Disorders of transsulfuration. In Scriver, C.R., Beaudet, A.L., Sly, W.S., Valle, D. (Editors). The Metabolic Basis of Inherited Disease, 6th edition, McGraw Hill Book Co., N.Y. 1987, in press
3. Gahl, W.A., Bernardini, I., Finkelstein, J.D., Tangerman, A., Martin, J.J., Blom, H.J., Mullen, K.D., and Mudd, S.H. Transsulfuration is an adult with hepatic methionine adenosyltransferase deficiency. submitted J. Clin. Invest. 5/11/87

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00940-06 LGCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Methionine Biosynthesis in Higher Plants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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0

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- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Investigation of the pathways for biosynthesis of phosphatidylcholine has been extended to two plants in addition to the Lemna previously studied, and to rat liver and hepatoma cells. Especial attention was focussed upon the role of phosphoethanolamine derivatives by use of methods which had demonstrated the participation of such derivatives in Lemna. Surprisingly, each of the plant tissues uses different steps to carry out the methylation reactions involved, although methylation of phosphoethanolamine appears to be the committing step common to each plant. Choline, or a metabolite thereof, markedly down-regulates entry of methyl groups into the network of methylated ethanolamine derivatives in each of the three tissues.

Rat liver uses a pathway which is again different in which all three methylations take place at the phosphatidyl-base level. In hepatoma cells it was demonstrated that entry of methyl groups into this pathway is regulated also by the availability of choline.

Project Description:

During the past year we have continued our investigations of the biosynthesis of phospholipids. Our earlier studies in this area, reported in previous annual reports, had demonstrated that Lemna utilizes a pathway for formation of phosphatidylcholine different from that previously accepted for both plants and animals. To assess the biological generality of this new pathway, we have now studied two additional higher plants, and reexamined liver using our new techniques. Surprisingly, the pathways utilized for this major biosynthetic process appear to be different in each of the four systems examined.

The plant systems have each been studied by a number of techniques. These include:

(a) Measurement of the rates of transfer of radioactively labeled methyl groups originating in methionine to methylated ethanolamine derivatives of three classes: the free bases, methylethanolamine (MEA), dimethylethanolamine (DMEA), and the trimethyl derivative, choline; the phosphate esters of these bases, i.e. phospho-bases (abbreviated as P-MEA, P-DMEA, and P-choline, respectively); and the phosphatidyl derivatives of the same bases, i.e. phosphatidyl-bases (abbreviated as phtd-MEA, phtd-DMEA, and phtd-choline). In these studies Lemna, or tissue-cultured cells of soybean or carrot were exposed continuously under unperturbed growth conditions to a tracer dose of methionine labeled with tritium in the methyl group. Exposure times were short, from 0.6 to 15 minutes in various experiments. The plant materials were harvested and rinsed rapidly, and homogenized at -60 degrees. Each of the methylated ethanolamine derivatives mentioned above was purified and the amount of tritium in each determined.

(b) Assay in cell-free extracts of enzyme activities catalyzing the following reactions:

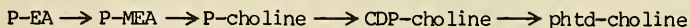
- (1) Transfer of methyl groups from S-adenosylmethionine to phospho-ethanolamine (P-EA), P-MEA, or P-DMEA.
- (2) Transfer of methyl groups from S-adenosylmethionine to phosphatidyl-ethanolamine (phtd-EA), phtd-MEA, phtd-DMEA.
- (3) Transfer of the cytidyl moiety of CTP to P-MEA, P-DMEA, or P-choline, forming the respective CDP-bases.

(c) Experiments similar to those described under (a), but utilizing plant material which had been grown for at least several doublings in a high external concentration of choline. These studies were aimed at investigating possible regulatory effects of exogenous choline.

The major results with the respective plants, and our interpretations of these results are as follows:

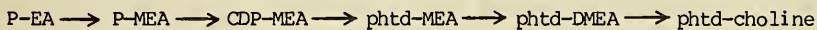
(a) Lemna. This plant rapidly incorporates methyl groups into P-MEA, P-DMEA, and P-choline. Radioactive methyls appear in phosphatidyl-bases only after a marked initial lag. For example at earliest times, about 100 times as much radioactivity has entered the phospho-bases as the phtd-bases. Radioactivity eventually accumulates almost entirely in phtd-choline, but the partially methylated phosphatidyl derivatives, phtd-MEA and phtd-DMEA, never acquire more than trivial amounts of radioactivity. The free bases at short times are

virtually unlabeled. Enzyme activities methylating P-EA, P-MEA, and P-DMEA were each demonstrated. Microsomes could methylate phtd-MEA and phtd-DMEA, but not phtd-EA. Cytidyl-transferases active with P-MEA, P-DMEA, and P-choline were demonstrated. These facts suggest that the major pathway is:



Minor pathways may occur via cytidyl transfers to P-MEA and P-DMEA, with subsequent formation of the phosphatidyl compounds, and their methylation to phtd-choline.

(b) Soybean. These cells rapidly incorporate methyl groups into P-MEA, but differ from Lemna in failing to incorporate detectable radioactivity into either P-DMEA or P-choline. Radioactivity appears rapidly also in phtd-MEA, phtd-DMEA, and phtd-choline, but not in any of the free bases. An enzyme methylating P-EA was demonstrated, but no activity was detected for methylation of P-MEA or P-DMEA. Microsomes were similar to those of Lemna, catalyzing methylation of phtd-MEA and phtd-DMEA, but not phtd-EA. Cytidyl transferase activities were similar to those of Lemna. These facts suggest that the major pathway is:



(c) Carrot. These cells resemble Lemna (and differ from soybean) in that they incorporate methyls rapidly into each of the three phospho-bases, P-MEA, P-DMEA, and P-choline. Conversely, carrot cells differ from Lemna (and resemble soybean) in that they incorporate methyls rapidly into each of the three phosphatidyl-bases, phtd-MEA, phtd-DMEA, and phtd-choline. Activities for methylation of P-EA, P-MEA, P-DMEA, phtd-MEA, and phtd-DMEA were demonstrated, but, again, none for methylation of phtd-EA. Cytidyl transferase activities were similar to those of both Lemna and soybean. These facts suggest that in carrot significant methylation occurs at both the phospho-base and the phtd-base levels. Quantitative interpretation is more difficult with these cells, but to emphasize the third possibility for a major pathway we tentatively suggest the following:

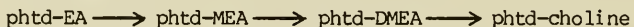


There are probably more or less significant cytidyl transfers to both P-MEA and P-choline also.

(d) With each tissue, growth in exogenous choline down-regulated entry of methyls into the total network of methylated ethanolamine derivatives. Such down-regulation amounted with Lemna to a 93% decrease; with soybean, to a 77% decrease; and with carrot, to a 99% decrease. The locus of this down-regulation may be placed with some confidence at the step which initially introduces methyl groups into this network. Methylation of P-EA, which we postulate is the committing step common to the three tissues examined would appear to be the best candidate for this regulation.

We have recently extended these studies to the synthesis of phtd-choline by rat liver and by hepatoma cells grown in tissue culture. Although it is generally accepted that mammals synthesize phtd-choline by successive methylations at the phosphatidyl-base level, a review of the evidence revealed that in the early experiments by Bremer and coworkers and Gibson and coworkers which are regarded as establishing the mammalian pathway, no attempt was made to directly detect the partially methylated P-bases after short incubations with methionine labeled in the methyl group. Further, in later experiments published in 1973, Salerno and Beeler (*Biochim Biophys Acta* 326, 325, 1973) reported that after intraportal injection of [³H₃C]methionine the specific activity of P-choline in rat liver rose rapidly to a maximum at 30 seconds after injection and then declined to a basal equilibrium before phtd-choline had reached one-half of its maximum value. These authors interpreted their results "to indicate the direct methylation of P-EA to P-choline". These observations have not been confirmed or refuted by subsequent publications. In addition, the possible regulation of phtd-choline synthesis by choline had not been settled for mammalian systems. For these reasons we decided to apply the methods which had successfully settled the analogous questions in plant tissues.

To study rat liver in as unperturbed a state as possible, two experiments were carried out: First, an intraperitoneal injection of [³H₃C]methionine with sacrifice of the animal 10 minutes after injection. Separate control experiments showed that, after intraperitoneal injection, total radioactivity in the liver increased almost linearly with time for at least 20 minutes. During the 10 minute experiment the liver was therefore exposed continuously to [³H₃C]methionine. Second, an intraportal injection over 30 seconds with immediate sacrifice of the animal. After the 10 minute experiment, 31% of the radioactivity in liver was converted to phtd-choline, 3% was recovered in phtd-DMEA, and 0.7% in phtd-MEA. No radioactivity (<0.02%) was detected in P-MEA or P-DMEA, with 0.05% in P-choline. After the 30 second experiment, 1.9% of total hepatic radioactivity was recovered in phtd-choline, 1.5% in phtd-DMEA, and 0.42% in phtd-MEA. No radioactivity (<0.004%) was detected in P-bases. These results provide strong support for the conclusion that in rat liver the pathway is:



With hepatoma cells, the relative proportion of methionine methyl converted to phtd-choline was much less, about 0.3% in 24-hour experiments. Again, no indication that P-bases participate as intermediates in the methylation process was obtained. However, it was possible to find conditions for these cells under which their growth was limited by external choline. After several days of exposure to such conditions, the rate of entry of methionine methyls into the phtd-bases was increased about 3-fold, demonstrating a considerable degree of regulation of the methylation pathway by choline, or a metabolite thereof.

Project No. Z01 MH 00940-06 LGCB

Publications:

Mudd, S.H., Datko, A.H.: Phosphoethanolamine Bases as Intermediates in Phosphatidylcholine Synthesis by Lemna. Plant Physiol., 82, 126-135 (1986)

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00942-06 LGCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

Biochemical Reactions in Mammalian Cell Chemotaxis

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	G. L. Cantoni	Chief, Laboratory of General and Comparative Biochemistry	LGCB NIMH

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0.5

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- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

Chemotaxis by the RAW264 mouse macrophage cell line was inhibited by 3-deazaadenosine but not by 3-deazaaristeromycin. A search for biochemical reactions inhibited by 3-deazaadenosine but not by 3-deazaaristeromycin has revealed that only one reaction, the synthesis of a small number of proteins identified after separation by two-dimensional polyacrylamide gel electrophoresis, has the necessary inhibitor specificity for involvement in the 3-deazaadenosine-sensitive step of chemotaxis. A study with several adenosine analogs showed a correlation between inhibition of chemotaxis and inhibition of the synthesis of a common subset of proteins. These analogs also inhibited the synthesis of polyadenylated mRNA, leading us to postulate that incubation of cells with 3-deazaadenosine inhibits methylation reaction(s) required for the formation of functional mRNA coding for one or more proteins required for chemotaxis.

Experiments to identify attractant-specific proteins have been limited because chemically defined attractants for RAW264 cells have not been available. This problem has been overcome by the isolation of a stable cell hybrid from a fusion between human leukocytes and a thioguanine-resistant RAW264 cell line. The hybrid expressed functional genes for chemotaxis to fMet-leu-phe, a commercially available synthetic attractant. Binding of fMet-leu-phe to hybrid cell membranes indicated that the binding constant was 2 nM and each cell had an average of 1200 receptors. In addition to chemotactic receptors, one or more guanine nucleotide binding proteins are required for chemotaxis by RAW264 and the hybrid cells. This conclusion is based on the observation that chemotaxis of either RAW264 or hybrid cells is inhibited upon incubation of the cells with either cholera toxin or pertussis toxin. In all cases entry of the toxin is required and there is a correlation between toxin-catalyzed ADP-ribosylation of a guanine nucleotide binding protein and the inhibition of chemotaxis. Although both cholera toxin and pertussis toxin affect cAMP levels, elevated cAMP levels per se do not inhibit chemotaxis. By immunochemical and electrophoretic techniques, the pertussis toxin substrate involved in chemotaxis has been identified as Ni-2, a protein that is also found in brain.

Other Investigators:

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G. Milligan	Assistant Professor, Univ. of Glasgow	
T.M. Caryk	Chemist	LGCB NIMH
L. Harvath	Research Microbiologist	DBBP FDA

The important discovery in this laboratory that chemotaxis by a macrophage cell line is specifically inhibited by 3-deaza-AdoHcy has allowed us to assess the significance of certain biochemical reactions in macrophage chemotaxis. Our conclusion was based on the finding that RAW264 chemotaxis is inhibited by 3-deazaadenosine but not by 3-deazaaristeromycin, and a search was initiated for a biochemical reaction that also showed this inhibitor specificity.

The synthesis of phosphatidylcholine by methylation of phosphatidylethanolamine, the release of arachidonic acid when cells are incubated with EAMS (endotoxin-activated mouse serum, an attractant for mouse macrophages), methylation of the lysine and arginine residues of protein, and protein carboxymethylation were all inhibited by both 3-deazaadenosine and 3-deazaaristeromycin. From these studies we conclude that none of these reactions are required for chemotaxis by RAW264 cells.

In contrast, the synthesis of a small number of proteins, identified after separation by two-dimensional polyacrylamide gel electrophoresis, does show the necessary inhibitor specificity for involvement in RAW264 chemotaxis. Quantitation of 100 of the more prominent proteins on the gels by computerized densitometry showed that in cells treated with 3-deazaadenosine the synthesis of approximately 10% of the proteins was inhibited by more than 50%, whereas in cells treated with 3-deazaaristeromycin the synthesis of these proteins was not significantly inhibited. The correlation of the inhibition of a subset of proteins with the inhibition of chemotaxis was strengthened by the finding that other inhibitors of chemotaxis inhibited the synthesis of the same subset of proteins. These inhibitors are 3-deoxyadenosine and the combination of erythro-9-(2-hydroxy-3-nonyl)adenosine (EHNA), adenosine and homocysteine. A common feature of the inhibitors of chemotaxis described above is that they all can inhibit the synthesis of functional mRNA. In this regard, we have also found that inhibitors of protein synthesis and translation, such as cycloheximide, puromycin and actinomycin D, inhibit chemotaxis.

We have proposed as a working hypothesis that treatment of RAW264 cells with 3-deazaadenosine, 3'-deoxyadenosine, and the combination of EHNA, adenosine and homocysteine inhibits the synthesis of functional mRNA coding for one or more chemotactic proteins. In support of this hypothesis, we have found that 3-deazaadenosine is a more potent inhibitor of polyadenylated mRNA than 3-deazaaristeromycin and that AdoHcy and 3-deazaAdoHcy do not inhibit in vitro translation.

Time-lapse video cinematography shows that motility and EAMS-induced morphological changes are similar in 3-deazaadenosine-treated and control cells. These observations suggest that in cells treated with 3-deazaadenosine, signal processing after attractant binding to the chemoreceptor is inhibited.

Additional studies to examine directly the effects of 3-deazaadenosine on attractant binding or to investigate the steps in signal transduction have been hindered by the lack of chemically defined attractants. The attractants described for RAW264 cells, EAMS and LDCF (lymphocyte-derived chemotactic factor), are both complex molecular mixtures with multiple biological activities. On the other hand, human monocytes and neutrophils are known to exhibit chemotaxis to FMLP (N-formylmet-leu-phe), a commercially available synthetic attractant. For these reasons hybrid cells were isolated from fusions between human leukocytes and thioguanine-resistant RAW264 cells, and some of the hybrids exhibited chemotaxis to FMLP and structurally related N-formylpeptides. The WBC264-9 cell line has been cultured for more than 6 months without loss of chemotaxis to FMLP demonstrating that a stable cell line has been obtained.

Chemotaxis of WBC264-9 and human leukocytes to FMLP are similar in several respects. The concentrations of N-formylpeptides that elicit the optimal chemotactic response in WBC264-9 cells are similar to the optimal chemotactic concentrations reported for human leukocytes. WBC264-9 migrates more quickly to FMLP than to EAMS, and the time course of WBC264-9 migration to FMLP is similar to that of human leukocytes. It also appears that WBC264-9 chemotaxis to FMLP and to EAMS may be regulated independently.

However, a study of the binding of radiolabeled FMLP to WBC264-9 cells indicated that WBC264-9 cells contained fewer receptors than human leukocytes do, although it should be noted that the receptor number is sufficient for chemotaxis. To reduce the technical problem of nonspecific binding, the number of receptors on membrane preparations was determined. From this data it was calculated that there are approximately 1200 receptors per WBC264-9 cell, compared to reported values from 2000 to 120,000 per cell for human leukocytes. The apparent dissociation constant for FMLP binding to WBC264-9 membranes was 2 nM, in agreement with values reported for the high affinity site of human leukocytes. It has been proposed that the high affinity receptors for FMLP mediate chemotaxis.

Studies on the human FMLP receptor have been carried out in collaboration with Dr. L. Harvath. Our laboratory's principal contribution has been the preparation and analytical determination of chemical derivatives of FMLP. These studies have shown that human monocytes exhibit chemotaxis for both FMLP sulfoxide and sulfone, whereas human neutrophils do not exhibit chemotaxis to either of the oxidized peptides. In contrast, both human neutrophils and monocytes migrate to FMLP, and both cell types generate superoxide anion, secrete enzymes and polarize when stimulated with FMLP, FMLP sulfoxide or FMLP sulfone. These data suggest that the FMLP receptor complex or chemotaxis transduction mechanism is different in human neutrophils and monocytes.

In collaboration with Dr. Harvath, we have also developed flow cytometric procedures that allow us to determine the subpopulation of leukocytes in whole blood that bind a fluorescent FMLP derivative and to determine the rate of binding of fluorescent FMLP to human neutrophils.

In addition to chemotactic receptors, one or more guanine nucleotide proteins (N-proteins) are required for chemotaxis by RAW264 and WBC264-9 cells. This

conclusion is based on the observation that chemotaxis of either RAW264 or WBC264-9 cells is inhibited upon incubation of the cells with either cholera toxin or pertussis toxin. In all cases entry of the toxin is required and there is a correlation between toxin-catalyzed ADP-ribosylation of an N-protein and the inhibition of chemotaxis.

Although both cholera toxin and pertussis toxin can affect cAMP levels, our evidence indicates that cAMP is not involved in chemotaxis. This was shown by elevating cAMP with either isoproterenol or forskolin to levels comparable to those achieved with cholera toxin. Chemotaxis of cells treated with isoproterenol or forskolin was not inhibited, showing that increased levels of cAMP per se do not inhibit chemotaxis.

In agreement with observations in several other laboratories, we found that the major membrane protein ADP-ribosylated by cholera toxin is distinct from that ADP-ribosylated by pertussis toxin. This was shown by the different electrophoretic mobilities of the proteins and the difference in the nucleotide specificity of the ADP-ribosylation reactions. However, under certain conditions, cholera toxin also appeared to ADP-ribosylate a membrane protein with a molecular weight similar or identical to the substrate for pertussis toxin.

Further immunochemical studies that employed a battery of antisera specific for several pertussis toxin substrates indicated that RAW264 cells have only one major pertussis toxin substrate identified as N_1-2 . A similar protein was also identified in bovine brain. It is likely that N_1-2 is the guanine nucleotide binding protein that couples chemotactic receptors to an effector protein such as phospholipase C or ion channels.

Significance of Biological Research to the Program of the Institute:

Several reports have shown that stress-induced neuropeptides modulate immunological activities and that leukocytes have receptors for beta-endorphin and other neuropeptides. Chemotaxis is an important component of the immunological response, and it has been shown that human monocytes exhibit chemotaxis to met-enkephalin and beta-endorphin. Injection of beta-endorphin into the rat cerebral ventricle results in the immigration of macrophage-like cells, indicating that chemotaxis to beta-endorphin can occur in vivo. Identification of the steps involved in chemotaxis would provide a basis for the development of strategies to counteract stress-induced immunological dysfunction.

Guanine nucleotide binding proteins are important components of signal transduction by both hormones and chemoattractants. Brain is also one of the richest sources of guanine nucleotide binding proteins, suggesting that these proteins may be important regulators of brain function. Studies of interactions between receptors, guanine nucleotide binding proteins, and various effector systems, such as adenylate cyclase, should improve our understanding of signal transduction mechanisms in cells.

Mammalian cell chemotaxis is also important in the development of the nervous system, inflammation and wound healing, and chemotaxis is a behavioral response

at the cellular level. Studies of bacterial chemotaxis from the laboratories of Koshland and Adler have shown that bacteria have "memory" and adapt to their environment, and progress has been made in explaining these concepts in molecular terms. The mammalian cell line model for chemotaxis that we have developed provides a mammalian system to test concepts developed from bacterial chemotaxis and to study the biochemical reactions involved in signal transduction.

Proposed Course of Research:

Future work will be directed toward the identification of biochemical components of chemotaxis. These problems will be approached by a combination of biochemical and molecular biology techniques.

Publication:

Aksamit, R.R.: A human-mouse hybrid cell line that stably expresses chemotaxis to N-formylmethionyl-leucyl-phenylalanine. Biochem. Biophys. Res. Commun. 138: 1001-1008, 1986.

Patent:

Aksamit, R.R.: A human-mouse hybrid cell line expressing monocyte-macrophage properties, ref E-404-85; patent pending.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00943-06 LGCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathways of Methionine and Threonine Metabolism and Their Control in Higher Plants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I.	J. Giovannelli	Research Chemist	LGCB NIMH
	S.H. Mudd	Chief, Section on Alkaloid Biosynthesis	LGCB NIMH
	A.H. Datko	Biologist	LGCB NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Alkaloid Biosynthesis

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

1.2

PROFESSIONAL:

1.2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

At least 80% of the total activity of aspartokinase in crude extracts of *Lemna paucicostata* was inhibited by lysine, with the remaining activity inhibited by threonine. Inhibition by lysine was synergistically increased by S-adenosylmethionine, which by itself had no effect. Inhibition by lysine and threonine was additive, not cooperative. Aspartokinase activity extracted from *Lemna* was an order of magnitude greater than that reported by other workers for other plant tissues, and in large excess (approximately 20-fold) of the in vivo requirements for synthesis of the aspartate family of amino acids. Either lysine-sensitive or threonine-sensitive aspartokinase activities alone can support the combined in vivo flux into the aspartate family of amino acids. Severe inhibition of both forms of enzyme activity was required to reduce this flux below the normal requirement. No evidence was obtained for repression/derepression of aspartokinase in plants grown with amino acids of the aspartate family. A major conclusion from these combined data is that, contrary to suggestions of other workers, the step catalyzed by aspartokinase does not appear to be the overall rate-limiting one for entry of 4-carbon units into the aspartate pathway. Further, the findings confirm the absence of major "channeling" of lysine-sensitive or threonine-sensitive aspartokinases into separate biosynthetic branches of the aspartate family. The findings also help explain why little, if any, feedback regulation of threonine synthesis occurs in plants supplemented with threonine alone, while complete feedback regulation of threonine synthesis occurs in plants supplemented with both threonine and lysine.

Project Description:

Aspartokinase catalyzes the first committing step in the synthesis of the aspartate family of amino acids (lysine, threonine, isoleucine and methionine), and has been suggested to act as the overall rate-limiting step in biosynthesis of these amino acids. The enzyme was studied in extracts of Lemna grown under control conditions or supplemented with one or more of the aspartate family of amino acids. The high-lights of these studies are:

(1) Using a sensitive and specific assay developed for these studies, aspartokinase was found to be comprised of two activities. At least 80% of the total activity of crude extracts was strongly inhibited by lysine, but not by threonine. Inhibition by lysine was synergistically increased by AdoMet, which by itself had no effect. The remaining (lysine-insensitive) activity was inhibited by threonine. Inhibition by lysine and threonine was additive, with no indication of concerted inhibition by these amino acids as reported in a number of bacteria and another species of Lemna (L. minor).

(2) Activity of aspartokinase extracted from Lemna was an order of magnitude greater than that reported by other workers for other plant tissues, and in large excess (approximately 20-fold) of the *in vivo* requirements for synthesis of the aspartate family of amino acids. Either lysine-sensitive or threonine-sensitive aspartokinase activities alone can support the combined *in vivo* flux into the aspartate family of amino acids. Severe inhibition of both forms of enzyme activity was required to reduce this flux below the normal requirement. Activities of lysine- and threonine-sensitive aspartokinase determined in gel-filtered extracts were similar for control plants and plants grown with a variety of supplements containing lysine, threonine, and methionine, indicating that aspartokinase is not subject to repression/derepression by amino acid products of the aspartate family. A major conclusion from these combined data is that, contrary to suggestions of other workers, the step catalyzed by aspartokinase does not appear to be the overall rate-limiting one for entry of 4-carbon units into the aspartate pathway. The adequacy of either lysine- or threonine-sensitive aspartokinase activities for normal flux requirements corroborates previous studies of labeling patterns obtained with [¹⁴C]homoserine that argue against "channeling" of lysine-sensitive or threonine-sensitive aspartokinase into separate biosynthetic branches of the aspartate family. Further, the finding that both lysine- and threonine-inhibited aspartokinase activities must be severely inhibited to cause significant reduction of flux through the aspartokinase step helps explain why little, if any, feedback regulation of threonine synthesis occurs in plants supplemented with threonine alone, while complete feedback regulation occurs in plants supplemented with both threonine and lysine.

Significance to Biomedical Research and the Program of the Institute:
Our combined in vivo and in vitro studies with Lemna reveal many unusual features in the regulation of synthesis of the aspartate family of amino acids. An important practical application of these studies is that we are now able to propose novel and logical strategies for improving the nutritional content of these amino acids in plants by genetic engineering.

Proposed Course of Future Research:

Regretably, we will not be able to pursue a number of intriguing avenues of research, since this project will be abolished with the Section in October, 1987.

Publications:

Giovanelli, J.: Cystathionine β -Lyase from Spinach. In W.B. Jacoby and O.W. Griffith (eds), Sulfur and Sulfur Amino Acids, Vol. 143, pp.443-449, 1987, Academic Press, New York.

Giovanelli, J.: Sulfur Amino Acids of Plants: An Overview. In W.B. Jacoby and O.W. Griffith (eds), Sulfur and Sulfur Amino Acids, Vol. 143, pp.419-426, 1987, Academic Press, New York.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02321-02 LCCB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

DNA Methylation and Gene E

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

P.I. G. L. Cantoni Chief, Laboratory of General LCCB
and Comparative Biochemistry

R. Razin Visiting Scientist, The Hebrew University
Jerusalem, Israel

Others: S. Agostini Guest Researcher LCCB
T. Gomi Visiting Fellow LCCB

COOPERATING UNITS (if any)

Department of Cellular Biochemistry, The Hebrew University, Hadassah Medical School, Jerusalem, Israel; Department of Human Biopathology, University of Rome, La Sapienza, Rome, Italy

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.5

PROFESSIONAL:

3

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

When Friend Erythroleukemia cells (FELC) are exposed to a variety of chemical agents capable of inducing terminal differentiation their DNA undergoes a genome-wide demethylation in the absence of DNA replication. Considerable evidence has accumulated to indicate that demethylation of specific genes, or of portion of specific regions of the DNA, is correlated with gene expression. The transient genome-wide demethylation observed during FELC differentiation must be an expression of the fact that the overall pattern of DNA methylation changes during differentiation with some genes becoming active in transcription and others becoming silent. The mechanism of DNA demethylation is completely unknown: theoretically, inhibition during at least two cycles of DNA replication of maintenance methylase, an enzyme capable of methylating hemimethylated DNA, could result in DNA demethylation and changes in the DNA methylation pattern. However the inhibition of maintenance methylase can not be involved in the genome wide, transient demethylation that is observed in the early phases of FELC differentiation, since this occurs in the absence of DNA duplication.

Project Description:

When Friend Erythroleukemia cells (FELC) are exposed to a variety of chemical agents capable of inducing terminal differentiation their DNA undergoes a genome-wide demethylation in the absence of DNA replication. Considerable evidence has accumulated to indicate that demethylation of specific genes, or of portion of specific regions of the DNA, is correlated with gene expression. The transient genome-wide demethylation observed during FELC differentiation must be an expression of the fact that the overall pattern of DNA methylation changes during differentiation with some genes becoming active in transcription and others becoming silent. The mechanism of DNA demethylation is completely unknown: theoretically inhibition of maintenance methylase, an enzyme capable of methylating hemimethylated DNA, during at least two cycles of DNA replication could result in DNA demethylation and changes in the DNA methylation pattern. However the inhibition of maintenance methylase can not be involved in the genome wide, transient demethylation that is observed in the early phases of FELC differentiation, since this occurs in the absence of DNA duplication.

In an attempt to elucidate the mechanism by which active demethylation takes place, we have considered three possible mechanisms: i) removal of the methyl group from 5-methylcytosine by direct demethylation; ii) removal of 5-methylcytosine and its replacement with cytosine through an enzymatic mechanism not previously described; iii) removal of a stretch of DNA that would include the 5-methylcytosine moiety by the conventional excision-repair mechanism. The first mechanism may be excluded a priori since it would require a reductive cleavage of a C--C bond by a biochemically unprecedented and improbable reaction. Experiments were designed to distinguish between these two mechanism. The results that we obtained indicate conclusively that transient DNA demethylation is due to a unique and novel mechanism whereby 5-methylcytosine is specifically replaced by cytosine. The ratio of 5-methylcytosine: cytosine in DNA extracted from cells 12 hours after induction is strikingly lower than that from untreated cells or from cells induced for 24 hrs. The timing of this transient demethylation indicates that the phenomenon occurs in the absence of DNA replication. The removal of 5-methylcytosine and its replacement by cytosine is specific both with regard to the base and its position in the sequence of deoxynucleosides in DNA. This conclusion is based on the demonstration that the cytosine residues that become incorporated into DNA in replacement of 5-methylcytosines were incorporated specifically into methylatable (or CpG) sites.

3-Deazaadenosine has been extensively studied in this lab for its ability to function as a substrate of AdoHcyase and give rise *in vivo* to a congener of Adenosylhomocysteine endowed with specific biochemical characteristics. In order to examine the relationship between biological methylation and the novel 5-methylcytosine replacement reaction the effect of the administration of 3-DZA on FELC cell differentiation was examined.

It was found that treatment of FELC with 3-DZA and homocysteine during the first 20 hours after induction with HMBA or DMSO will completely inhibit the expression of the differentiated state (measured at 72-96 hours). By contrast when treatment with 3DZA was delayed until 24 hours after induction

differentiation was not affected. The effect of 3DZA was specific (adenosine nor deazaaristeromycin were active had no effect) and required the presence of homocysteine, a result that indicates conclusively that the effect is mediated by adenosylhomocysteinase. The striking correspondence in the timing of the inhibition of differentiation produced by 3DZA and the replacement of 5-methylcytosine by cytosine adds weight to the hypothesis that this limited and specific modification of DNA structure is correlated with gene expression. Further work with intact cells is under way in order to delineate with greater precision the sequence of events that take place within 24 hours after induction and that commit the cells to differentiation. Hopefully these experiments will lead to studies in cell free systems and characterization of the enzyme mechanisms involved.

Significance of the Program to the Institute:

Biological methylation underlies many different physiological events. A role for biological methylation has been proposed for such diverse phenomena as memory and chemotaxis in bacteria, repair of cellular damage due to aging, fruit ripening, membrane and receptor function, synthesis and maturation of messenger RNA and gene expression.

In addition there is a growing body of experimental evidence that suggests that alteration in specific methylation reactions may be important in disease. The results from well controlled clinical studies have documented the efficacy of S-Adenosylmethionine in depression, in chronic arthritis and in liver diseases. The biochemical basis for these effects is entirely unknown and the search for suitable explanations for these pharmacological effects presents a formidable challenge.

All methylation reactions utilize S-Adenosylmethionine as the methyl donor and are inhibited by S-Adenosylhomocysteine. The great number and variety of reactions involving these intermediates has provided both an opportunity and an obstacle to the elucidation of the controlling role of methylation reactions in the different phenomena listed above. Continued efforts directed to unraveling the physiological significance and control of methylation reactions is fundamental to progress in this important area of research.

Publication:

None.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 MH 01037-19 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Role of the Cell Membrane in Cellular Organization: A Molecular Study

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	D. M. Neville, Jr.	Chief, Sec. on Biophy. Chem.	LMB, NIMH
Others:	T. H. Hudson	Staff Fellow	LMB, NIMH
	J. W. Marsh	Staff Fellow	LMB, NIMH
	K. Srinivasachar	Visiting Associate	LMB, NIMH
	K.-H. Jung	Visiting Fellow	LMB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Biophysical Chemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

5.3

PROFESSIONAL:

3.3

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The general aim of this project is to determine the chemical interactions and energetics such as membrane potential and pH and ion gradients which are involved in the insertion of proteins into cellular membranes and/or the translocation of proteins across cellular membranes. The events are studied from the initial receptor binding to the final physiologic response or pathological response in the case of toxins such as ricin, colicins, diphtheria and tetanus toxins. Utilizing basic data from such studies immunotoxins (toxins linked to monoclonal antibodies) are constructed to serve as a new class of pharmacologic reagents to eliminate unwanted cell types such as cancer cells or T-4 lymphocytes in AIDS infections, or to manipulate specific cells such as T cell subsets to correct imbalances which exist in autoimmune diseases which can affect the CNS such as multiple sclerosis and lupus and cause psychosis. In addition immunotoxins continue to prove useful in diminishing the incidence of graft-versus-host-disease following bone marrow transplantation and thus will also have utility in enzyme replacement therapy and organ transplantation.

Project Descriptions:

The general aim of this project is to determine the chemical interactions and energetics such as membrane potential and pH and ion gradients which are involved in the insertion of proteins into cellular membranes and/or the translocation of proteins across cellular membranes. The events are studied from the initial receptor binding to the final physiologic response or pathological response in the case of toxins such as ricin, colicins, diphtheria and tetanus toxins. Utilizing basic data from such studies immunotoxins (toxins linked to monoclonal antibodies) are constructed to serve as a new class of pharmacologic reagents to eliminate unwanted cell types such as cancer cells or T-4 lymphocytes in AIDS infections, or to manipulate specific cells such as T cell subsets to correct imbalances which exist in autoimmune diseases which can affect the CNS such as multiple sclerosis and lupus and cause psychosis. In addition immunotoxins continue to prove useful in deminishing the incidence of graft-versus-host-disease following bone marrow transplantation and thus will also have utility in enzyme replacement therapy and organ transplantation.

Major Findings:

Diphtheria toxin translocation across the cell membrane is driven by a plasma membrane voltage gradient or a pH gradient. In contrast, ricin translocation is not coupled to these gradients but is tightly coupled to the availability of ATP.

A tissue culture model system for studying the effects of tetanus toxin on the inhibition of neurotransmitter release has been developed utilizing NG-108 cells. The productive receptor appears to be a ganglioside present in very low amounts (500 molecules per cell).

Diffusion barriers to immunotoxin exist between the peritoneal cavity and the intravascular compartment. This enhances the effects of I.P. administered immunotoxins on tumor loads within the peritoneal cavity where 3-4 log kills are achievable. Irradiation of specific immune system subsets lying within the vascular compartment is more readily accomplished by using the intravascular route.

Significance to Biomedical Research and the Program of the Institute:

The identification of voltage gradients, pH gradients and ATP stores as the driving forces for protein toxin insertion and translocation prompts one to look for physiological insertions and translocation driven by the same forces. The toxins are thus model systems in which protein insertion into and translocation across membranes can be quantitatively studied. Protein insertion into membranes results in altered membrane functions. For example, differential protein insertion into neuronal membranes is believed to play an important role in establishing and maintaining neuronal synaptic connections.

The development of an animal model system for the evaluation of *in vivo* immunotoxin manipulation of T cell subsets is a first step in evaluating these agents for their therapeutic potential in the treatment of autoimmune diseases and AIDS.

Of particular interest to NIMH is the recently demonstrated association of serum and CSF autoantibodies directed at the carboxy terminus of ribosomal phosphoproteins and episodes of psychosis in patients suffering from systemic lupus erythematosus (Bonfa et al. 1987).

Of great promise is the possibility that immunotoxins directed at T4a⁺ T cells and macrophages could, early in the course of AIDS, eliminate the HIV infected cells before the virus spreads to cells whose eradication would prove harmful, i.e., neurons. It is also important to determine if the

maintenance CNS HIV infection (and resulting progressive dementia) requires a feeder population of easily infected cells, i.e., systemic T-4a⁺ macrophages and T cells.

Proposed Course:

Identification of the energy sources for toxin translocation permit toxin translocation to be studied in simple *in vitro* systems utilizing mixtures of cell fractions, cell "sap" and energy sources and inhibitors of these sources. Isolation of toxin enriched vesicles should provide the cellular components which document cellular sorting and packaging of proteins and provide an enrichment of the protein translocation machinery.

By using voltage sensitive dyes which report membrane potential changes via fluorescent changes, we hope to be able to map the insertion of arrays of membrane proteins both spatially and temporally in living cells following a variety of perturbations. State-of-the-art image processing hardware and software operating on digitized images is being assembled to perform this task. This is a new methodology which should be applicable to studying membrane insertions of pathological materials (toxins) and physiological insertions (ion conducting channels).

The development of immunotoxins for human *in vivo* use is being pursued by devising various strategies to reversibly block the toxin binding site of the immunotoxin. The site must be blocked outside the cell to achieve specificity but must be unblocked inside the cell where it is utilized for efficient translocation thus providing high efficacy to the reagent.

Immunotoxins constructed with intact diphtheria toxin, which have specificity and efficacy in mice, will be directed to eliminate helper and suppressor murine T cell subsets. The effects of these on the functioning of the immune system and the retention of immunologic memory will be assessed. As soon as immunotoxins are capable of achieving efficacy and specificity in humans are developed, the murine studies will be repeated in monkeys which share many of the same T cell subsets with humans. This will provide the necessary animal studies prior to clinical trials.

Publications:

Marsh, J.W. and Neville, D.M., Jr.: Kinetic comparison of ricin immunotoxins: Biricin conjugate has potentiated cytotoxicity. Biochemistry 25: 4461-4467, 1986.

Hudson, T.H. and Neville, D.M., Jr.: Temporal separation of protein toxin translocation from processing events. J. Biol. Chem. (in press).

Hudson, T.H. and Neville, D.M., Jr.: Enhancement of immunotoxin action: Manipulation of the cellular routing of proteins. In: (Frankel, A., Ed.) Immunotoxins, Martinus Nijhoff Publ., Boston (in press).

Marsh, J.W. and Neville, D.M., Jr.: Development of an immunotoxin with *in-vivo* efficacy for murine systems. NY Acad. Sci. (in press).

Neville, D.M., Jr.: Immunotoxins for *in vivo* therapy: Where are we? NY Acad. Sci. (in press).

Neville, D.M., Jr. and Marsh, J.W.: Methods for quantifying immunotoxin efficacy. In: (Frankel, A., Ed.) Immunotoxins, Martinus Nijhoff Publ., Boston (in press).

Marsh, J.W., Srinivasachar, K., and Neville, D.M., Jr.: Antibody-toxin conjugation. In: (Frankel, A., Ed.) Immunotoxins, Martinus Nijhoff Publ., Boston (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
 NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 MH 01035-19 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Process of Lysogeny

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	H. A. Nash	Chief, Sec. on Molecular Genetics	LMB, NIMH
Others:	P. Kitts	Visiting Associate	LMB, NIMH
	M. Bruist	Research Associate	LMB, NIMH
	C.-C. Yang	Visiting Fellow	LMB, NIMH

COOPERATING UNITS (if any)

Laboratory of Molecular Genetics, NICHD; Department of Microbiology, University of Illinois, Urbana, IL; and Cell Genetics Department, South San Francisco, CA

LAB/BRANCH

Laboratory of Molecular Biology

SECTION

Section on Molecular Genetics

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.75

PROFESSIONAL:

3.75

OTHER:

1.0

CHECK APPROPRIATE BOXES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have investigated the mechanism by which the DNA of bacteriophage lambda integrates into the chromosome of its Escherichia coli host. We studied the role of DNA homology between the recombining sequences and ruled out two plausible alternatives for this requirement. We also determined that the two recombination partners interact with recombination proteins very differently. The bacterial partner obtains its recombinase only by collision with a nucleoprotein assembly formed on the viral partner. The genes for a second recombination protein have been placed on an overexpression vector and thus enabled the synthesis of large quantities of active protein.

Objectives:

Rearrangement of genetic information by breakage and reunion of DNA sequences is an important feature of all living organisms. DNA rearrangements are used to alter the genetic content of a cell in a precise, controlled and potentially reversible way. The life cycle of retroviruses, the generation of components of the immune system and the expression of alternate genes all depend upon recombination between specific sites in DNA. This project has as its goal the elucidation of the molecular mechanism of a prototypical site-specific recombination: the integration of the DNA of bacteriophage lambda into the chromosome of its *E. coli* host. We want to know how specific recombination proteins can locate special sequences in DNA, bring them together and carry out a cycle of events that includes breaking the DNA, switching the broken strands to new partners and resealing of the new joints. Our understanding of this reaction not only serves as a model for understanding other members of its family but also a broad spectrum of complex biological processes that involve interactions between more than one protein species with multiple targets in DNA.

Major Findings:

Integration of the chromosome of bacteriophage lambda involves the interaction between two pieces of DNA - the viral attachment site, *attP*, and bacterial attachment site *attB*. These sites have in common a 15 base pair core sequence within which the crossover takes place. We have shown that the viral Int protein can cut DNA within the core to initiate strand exchange and can subsequently reseal the broken DNA. Each strand of DNA within the core is cut by Int at a unique place but the two cuts are separated by 7 base pairs. Others have shown that efficient recombination requires that this 7 base pair "overlap" sequence be identical in two attachment sites. It has long been speculated that this requirement reflects a mechanism of recombination in which double strand breaks are introduced into each attachment site, thereby generating breaks with protruding single stranded ends. The model asserts that recombination involves annealing of one end from each parent, a process that is expected to require that both parents have the identical overlap sequence. We have now proven that this model is wrong. Our test was based on *in vitro* Int-promoted crosses in which one attachment site is a heteroduplex. Specifically, we constructed sites in which the overlap region contains one or more non-complementary pairs. The double-strand break and annealing mechanism predicts that crosses with such heteroduplex sites should yield one completed recombinant and one broken site. Instead, we find that non-reciprocal recombination is uncommon and that the typical outcome of crosses involving a heteroduplex site is a reciprocal recombinant in which both products are resealed. Moreover, the occasional appearance of non-reciprocal products is explained by our finding that Int can cleave heteroduplex attachment sites after recombination is completed. Taken together, our data strongly indicate that lambda integrative recombination does not proceed by the homology-dependent annealing of cohesive ends. Alternatives that demand homology to enable attachment sites to come together are consistent with our results, as are models that invoke the initiation of recombination by the exchange of one strand for each parent (rather than a double strand break) followed by a homology-dependent migration of the resulting Holliday structure.

Heteroduplexes have also been used to tell us about the state of *attB* when it participates in a recombination reaction. Specifically, we want to know whether *attB* encounters *attP* when it is decorated with Int protein that it has obtained from free solution or whether it begins recombination as a naked piece of DNA, deriving its Int from the many copies that are tightly bound to *attP*. We had previously shown that heteroduplexes of an attachment site that, like *attP*, has multiple Int binding sites are efficiently cleaved by Int. This is because when Int cuts a single strand of the heteroduplex the broken ends can not be resealed due to lack of complementarity. Ultimately double strand breaks accumulate in the heteroduplex and become a permanent record for the binding of Int

protein. In contrast to sites like *attP* with multiple *Int* targets, *attB* heteroduplexes are scarcely cut by *Int*. This suggests that *Int* fails to bind efficiently to *attB*, an hypothesis supported by chemical protection studies. These footprinting experiments show that, although *attB* does have binding sites for *Int*, they are very weak and can not compete for *Int* protein with the strong sites on *attP* or even with those found in non-specific DNA. Although, cleavage of *attB* heteroduplexes by *Int* is inefficient, the reaction is dramatically stimulated by addition of *attP*. This cleavage reaction therefore provides a novel assay for the interaction between *attB* and *attP*, a device we have used to show that synapsis does not depend upon homology in the overlap region between *attP* and *attB*. This results rules out one of the two possibilities that we discussed above and strongly supports the remaining hypothesis, i.e., that the role for homology is in the branch migration of Holliday structures.

In addition to *Int*, the virus-encoded protein that carries out breakage and reunion, integrative recombination requires a host protein. This protein, which we named IHF (for integrative host factor) is of general interest. First, mutants of IHF show alterations in many processes that are important for *E. coli*, its plasmid and phages. Moreover, IHF protein has been directly implicated as an essential component of *in vitro* reactions as diverse as the initiation of transcription, the packaging of viral DNA, and the transposition of movable genetic elements. Second, IHF may represent a novel form of DNA sequence-specific binding protein that recognizes an assymetric sequence through contact with base pairs in the minor groove of DNA. To assist future structural and chemical studies of IHF we have constructed strains that overproduce the protein. IHF is composed of two dissimilar subunits, each containing about 100 amino acids. The genes for these two subunits lie far apart on the *E. coli* chromosome and are expressed under separate control mechanisms. We find that when each gene is overexpressed separately, the resulting polypeptides are either unstable or insoluble. By contrast, overexpression of both genes conjointly leads to the accumulation of large amounts of active IHF. Extracts of such cells provide the starting material for a rapid purification procedure that results in milligram quantities of apparently homogenous IHF.

Significance to Biomedical Research and the Program of the Institute:

Two features of lambda integrative recombination make it an important reaction to try to understand. First, during lambda integration DNA gets broken and rejoined into a novel arrangement. Related rearrangements of DNA are well known in molecular biology and more examples are being discovered at a rapid rate. It is widely believed, and some early successes support the notion, that lessons learned in one system will be applicable to many. Thus, our progress in delineating the role of homology during lambda integrative recombination and our analysis of the different way that two recombination partners interact with the recombinase will be widely appreciated by others studying both site-specific and homologous recombination in prokaryotic and eukaryotic systems. The second feature of integrative recombination that makes it a significant model system is the complexity of its protein-DNA interactions. It has been widely accepted that important steps in higher organisms such as turning gene expression on or off during development and activating origins of replication during cell division are multicomponent processes that involve the interaction of many proteins binding to many DNA sites. The integrative recombination reaction is one such multicomponent system in which the basic elements are well defined and the components have all been well characterized at the biochemical level. Thus, as we uncover the logic that underlies this reaction, we provide insight to a broad spectrum of molecular biologists.

Proposed Course:

The experiments refuting the cohesive end model for homology dependence in integrative recombination are completed and a paper describing the results has just been published. The use of heteroduplex substrates to analyze the state of *attB* in recombination is being prepared for publication. The major conclusion of this work is that *attB* enters into recombination as a naked piece of DNA and acquires its recombination protein not from solution but from its recombination partner. We would like to test this unusual proposal by preparing *attP* nucleoprotein complexes, freeing them from unbound protein and determining the kinetics with which such complexes recombine with *attB*. We also plan to modify *attP* and test which features of this multiprotein assembly are required for capture of *attB*. To study the structural features of IHF in more detail, we are exploiting our overproducer strain to generate large quantities of the pure protein. There has already been some initial success at obtaining crystals suitable for X-ray diffraction. We also plan to undertake a wide variety of footprinting studies to clarify the way in which IHF contacts DNA. These studies will be complemented by our planned measurement of the stoichiometry with which IHF binds to its specific site.

Publications:

Nash, H.A.: Virus-host interactions in site-specific recombination of bacteriophage lambda. Genetic Chemistry: The Molecular Basis of Heredity. Robert A. Welch Foundation Conference on Chemical Research. Vol. 29, Houston, Texas, 1985, pp. 285-296.

Gardner, J.F. and Nash, H.A.: Role of *Escherichia coli* IHF protein in lambda site-specific recombination: A mutational analysis of binding sites. J. Mol. Biol. 191: 181-189, 1986.

Richet, E., Abcarian, P., and Nash, H.A.: The interaction of recombination proteins with supercoiled DNA: Defining the role of supercoiling in lambda integrative recombination. Cell 46: 1011-1021, 1986.

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Kitts, P.A. and Nash, H.A.: Homology dependent interactions in phage λ site-specific recombination. Nature (in press)

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 MH 02228-03 LMB

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Neurobiology of *Drosophila*

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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 Others: K. Weber Graduate Student Harvard Univ.

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TOTAL MAN-YEARS:

1.25

PROFESSIONAL:

0.25

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We have isolated mutants of the fruit fly *Drosophila melanogaster* that show an altered response to general anesthetics. We have mutagenized flies with ethylmethane sulfonate and found amongst their offspring mutants that are hypersensitive or resistant to halothane. These mutants form the starting point for a genetic and a molecular biological study of the mechanism by which anesthetics interfere with pain and consciousness.

Objectives:

Genetic analysis has been a successful strategy for unraveling complex processes in a variety of organisms. In recent years there has been significant success in using genetic techniques to illuminate developmental, physiological, and biochemical aspects of the nervous system of the fruit fly, *Drosophila melanogaster*. The goal of this project is to extend such studies into previously unexplored territory. We are especially interested in using genetics to analyze the mechanism of general anesthesia. To this end, we have undertaken the isolation of mutants with altered sensitivity to anesthetics. Amongst such mutants should be some that change the putative target of the agent. Analysis of these mutants by physiological and molecular biological techniques should help in understanding both the anatomic and biochemical basis of consciousness and the perception of pain.

Major Findings:

Success at isolating mutants depends on two components: efficient mutagenesis and a sensitive screening procedure. In the past year we have learned how to successfully apply a classical chemical mutagenesis protocol. From the frequency of sex-linked lethal mutations and easily scored eye color variants in our mutagenized population, we estimate that each locus in the genome of these offspring has a one in a thousand chance of being mutated. Since *Drosophila* is estimated to have five thousand to ten thousand genetic loci, one expects that, if a particular gene is not essential for survival, then screening twenty thousand offspring should yield a handful of mutations in that gene. Because of the ease of screening for recessive mutations, we have focused our efforts on sex-linked genes. Mutagenized males are crossed with attached-X virgin females and the subsequent generation is screened en masse for the response to anesthesia.

To screen the offspring, we have adapted a device introduced by K. Weber (Harvard University) to quantitate the response of flies to intoxication by alcohol. This "inebriometer" consists of a vertical glass column fitted with nylon mesh baffles that impede the fall of partially anesthetized flies. We have learned how to provide a reliable, constant dose of volatile anesthetic to the inebriometer and have screened our mutagenized population for an altered response to anesthetic.

By focusing on the first and last flies to exit the column, we identify potential hypersensitive and resistant mutants. Those flies that show a consistent response upon retesting are individually mated to generate a clonal line. We have now identified several such lines that are reproducibly hypersensitive or resistant to a fixed concentration of the clinical agent, Halothane. In most cases, only male flies are affected, as expected for a recessive sex-linked mutation. The severity of the alteration to anesthesia varies amongst the different lines from mild to moderate. We conclude that the anesthetic response is amenable to a genetic analysis.

Significance to Biomedical Research and the Program of the Institute:

Analysis of the conscious state is a subject of interest to neurobiologists. One wants to know what anatomic structures are essential and what neurophysiological mechanisms contribute to awareness. Anesthetics have long been recognized as potentially useful reagents in investigating the conscious state. However, their wide distribution in the brain has hindered the formulation of decisive tests. Identifying the genes that control or encode anesthetic targets should help in this endeavour. The distribution of gene products in the brain could provide information on the anatomical basis of the conscious state and the nature of the gene products could tell us about important cellular mechanisms. Our success in isolating mutants with altered sensitivity to anesthetics represents an important first step in this analysis.

Proposed Course:

We will continue to collect more anesthetic mutants; this exercise is potentially useful up to the point where one begins to recover the same mutants repeatedly, i.e., until one saturates the genome. However, we already have enough mutants on hand to begin a more detailed pharmacological and genetic characterization. We want to quantify the degree of sensitivity to Halothane, test the response to other anesthetics, and devise tests of the response to anesthesia that will be applicable to single flies. The latter is important for the genetic characterization of the mutants. We want to map them precisely, determine their genetic complementation both with wild-type and other mutant alleles, and begin the search for genetic suppressors.

PERIOD COVERED
 October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
 The Biochemical Basis of Peptide Receptor Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	W. A. Klee	Chief, Sec. on Regulatory Proteins	LMB, NIMH
Others:	D. L. Newton	Staff Fellow	LMB, NIMH
	J.-Y. Ye	Visiting Associate	LMB, NIMH
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither

(a1) Minors

(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the past year we have continued our studies on reconstituted opiate receptors in purified systems. Purified G-proteins and adenylate cyclase were reconstituted into liposomes from detergent solutions by dialysis in the presence of phospholipids. In the reconstituted vesicles adenylate cyclase activity is stimulated by Gs and the stimulated activity is inhibited by Gi or by the beta-gamma subunit complex of bovine transducin. Further progress has been made in our efforts to obtain useful amounts of purified opiate receptors, and in characterizing the physical and biochemical properties of G-proteins.

We have identified a monoclonal antibody, directed against a defined region of the amino acid sequence of bovine transducin, which also recognizes opiate receptors from NG108-15 neuroblastoma x glioma hybrid cells. A peptide corresponding to the epitope of the antibody inhibits precipitation of opiate receptors by the antibody. A peptide from the homologous region of the porcine brain muscarinic receptor not only blocks the antibody, but also activates a number of G-proteins both in membranes and in solutions of the purified proteins. We have thus identified the signal transmitting domain of G-protein coupled receptors.

Project Description and Major Findings:

Receptors on the cell surface, such as those for the opiates, are coupled to enzymes, such as adenylate cyclase, on the inside of the membrane via the mediation of GTP-binding regulatory proteins (G-proteins). In this manner, information is transmitted to a cell from neighboring cells and from the fluid environment. Our goal is to dissect this system into its component parts, and study each of the proteins and other components of the system both in isolation and as a reconstituted functional entity. In the past few months, the Section has achieved a major breakthrough that has resulted in identification of the receptor domain that activates G-proteins and the demonstration that peptides corresponding to this domain stimulate G-protein activity in the absence of any other substances.

We have chosen to concentrate upon opiate receptors in the cultured neuronal cell line, NG108-15. These cells are richly endowed with opiate receptors of a single type, namely δ . The receptors were shown to be coupled, as inhibitors, to adenylate cyclase both in these cells and in brain tissue. Activation of the receptors with opiates or opioid peptides reduces cellular cyclic AMP levels and thereby lowers the extent of phosphorylation of many cellular enzymes. In analogy to the addictive process, the cells become tolerant to and dependent upon opiates after prolonged exposure. This adaptive process is due to a gradual increase in adenylate cyclase activity which serves to maintain normal cyclic AMP levels in the continued presence of opiates. With opiates such as morphine, adaptation occurs in the absence of changes in receptor number. Other opioids, such as the enkephalins, produce receptor down-regulation as well as increased adenylate cyclase activity upon chronic exposure.

We have over the past few years developed procedures for the solubilization of receptors from membranes by extraction with the zwitterionic detergent CHAPS, and have isolated affinity labeled opiate receptors in a homogeneous state. Such receptors, because of the presence of covalently linked opiates are more useful for structural than for functional studies. They have proven to be particularly good tools for antibody screening experiments as well.

All receptors share two essential properties: they bind ligands, and transmit the information of whether or not an activating ligand (agonist) is bound. Many receptors send information to one of several GTP-binding regulatory proteins (G-proteins) which have very similar amino acid sequences. This family of G-proteins includes at least 3 types of Gi, Go, and transducin. Receptors coupled to these proteins include, among others, opiate, muscarinic and bradykinin receptors and the photon receptor, rhodopsin. Activation of G-proteins by agonist occupancy of these receptors ultimately results in activation, or inhibition, of one of several enzymes including phospholipase C, cyclic GMP phosphodiesterase and adenylate cyclase. We reasoned that receptors of this class, which must all interact with very similar regulatory proteins, might share structural features responsible for these interactions.

The availability of a battery of monoclonal antibodies directed against defined regions of rhodopsin (developed by Dr. Paul Hargrave and his collaborators at the University of Florida), allowed an experimental test of the hypothesis. For this test we used opiate receptors from NG108-15 neuroblastoma X glioma hybrid cells specifically substituted with the synthetic opiate [3H]-3-methylfentanylisothiocyanate (superFIT). We found that one of the 46 anti-rhodopsin monoclonal antibodies tested also recognizes opiate receptors. The epitope against which this antibody is directed corresponds to a cytoplasmic segment of the rhodopsin molecule immediately following the seventh (putative) transmembrane helix. A peptide corresponding to this region, rhodopsin 310-321, blocks interaction of the antibody with both rhodopsin and opiate receptors. An amidated peptide corresponding to the homologous region of the porcine brain muscarinic receptor, residues 422-431 (N10L) also blocks the antibody. Interestingly, this peptide activates Gi function in S49

cell membranes, where it inhibits adenylate cyclase and stimulates low K_m GTPase. The peptide also activates G-proteins in membranes of NG108-15 cells. In solution and at concentrations below about 200 μM , the peptide stimulates the GTPase activity of both purified transducin and purified Go. These experiments suggest that N10L corresponds to the receptor domain directly responsible for information transmission and show that it can work in a completely defined system. Thus, many experiments can now be performed using only purified proteins and peptides that should greatly clarify the mechanism of receptor action. As more receptor sequences become available much will be learned using this approach about receptor specificity and selectivity.

Receptor preparations which have not been irreversibly modified are needed for functional reconstitution studies. To this end, we have prepared several affinity columns consisting of opiates covalently linked to cross-linked agarose beads to which opiate receptors bind. In combination with lectin affinity chromatography this type of procedure has allowed the partial purification of opiate receptors from NG108-15 cell membranes. The yields obtained in these procedures have, so far, been low when receptors are assayed by opiate binding activity. One reason for the apparently low yields has recently been discovered by Dr. Newton, who has found that the solubilized receptors are composed of a mixture of high and low affinity molecules. The low affinity receptors had been missed up until now because of technical problems in their assay.

Several GTP-binding regulatory proteins have been characterized, but, with the exception of that of G_s , the functions of these proteins have not been clearly established. The G-proteins are members of a closely related family and are each composed of three subunits: α , β , and γ . The major differences in structure among the several G-proteins are found in the α subunits. The β subunits are all identical or nearly so and the β - γ subunit complexes of the most diverse of the G-proteins have been found to substitute for one another. We have purified two such proteins, G_i and G_o from bovine brain and prepared antibodies which recognize the α subunits of one or the other protein. It has recently become clear that there are at least three forms of G_i , two of which are present in brain. Liver, on the other hand, contains primarily a single form of G_i and hardly any G_o . We have therefore purified the liver protein and are in the process of characterizing it better. This material will significantly aid our studies of receptor signaling domains.

The G-proteins are present in fairly low amounts in most tissues. Even in brain, which is fairly rich in G-proteins, the presence of at least 4 such proteins complicates isolation of really pure samples. In some of our studies we therefore use transducin, the G-protein of retina, which is easily available in mg amounts as a homogenous protein. Dr. Ye has developed methods for the study of the isolated subunits of transducin. Separation of the α from the $\beta\gamma$ subunits of the protein is readily accomplished in good yield and with retention of native properties. Dr. Ye has studied the physical properties of the isolated subunits by measuring fluorescence and circular dichroism both under native and denaturing conditions. In addition, he has demonstrated that the subunits contain both disulfide and sulfhydryl functions. This unusual circumstance may be important to the function of the protein and certainly complicates its study. For example, separation of β from the γ subunit requires prior denaturation in urea or guanidine hydrochloride. Reconstitution of the two to a functionally active entity requires a reversal of the denaturation, presumably under controlled redox conditions. In order to minimize problems associated with disulfide interchange and other slowly reversible processes, we have developed an HPLC separation of the two subunits which can be accomplished at low temperatures and within 12 minutes. These studies will not only increase our understanding of transducin as a prototypic G-protein, but also serve as paradigms for the study of the other, less readily obtained, members of the family.

Significance to Biomedical Research and the Program of the Institute:

A major problem in biology is understanding the mechanism of signal-response coupling across cell membranes. Cells communicate with one another and with their environment largely through chemical messengers which are sensed by cell surface receptors and thereby elicit other chemical changes within the cell. The opiates, and related substances, are important transmitters of information in the nervous system. An understanding of how brain cells transmit and use such information is essential to the design of rational therapy for mental illness.

Proposed Course:

We plan to continue our efforts to understand the molecular basis of signal transduction, with particular emphasis on opiate receptor and related mechanisms. In the next year we hope to be able to use our newly discovered insight into the nature of the signaling domain of receptors to learn more of the nature of receptor G-protein activation mechanisms. The approach may also prove useful to elucidate those aspects of receptor structure which determine affinity for particular G-proteins. It does not seem unreasonable to hope that a completely synthetic receptor may be prepared that reproduces not only the signaling properties of hormone action but also the high affinity interaction with specific G-proteins as well.

Publications:

Burke, T.R., Jr., Jacobson, A.E., Rice, K.C., Silverton, J.V., Simonds, W.F., Sreaty, R.A., and Klee, W.A.: cis-(+)-3-methylfentanyl isothiocyanate, a potent site-directed acylating agent for δ opioid receptors. Synthesis, absolute configuration, and receptor enantioselectivity. J. Med. Chem. 29: 1087-1093, 1986.

Lessor, R.A., Bajwa, B.S., Rice, K.C., Jacobson, A.E., Sreaty, R.A., and Klee, W.A.: Potential irreversible narcotic antagonist-based ligands derived from 6,14-*endo* ethenotetrahydrooripavine with 7-(Methylfumaroyl) amino, (Bromacetyl)amino, or isothiocyanate electrophiles: Chemistry, Biochemistry and Pharmacology. J. Med. Chem. 29: 2136-2141, 1986.

Schönenberger, B., Jacobson, A.E., Brossi, A., Sreaty, R., Klee, W.A., Flippen-Anderson, J.L., and Gilardi, R.: Comparison of (-)-Eseroline with (+)-Eseroline and dihydroseco analogues in antinociceptive assays: Confirmation of rubreserine structure by X-ray analysis. J. Med. Chem. 29: 2268-2273, 1986.

Milligan, G., Sreaty, R.A., Gierschik, P., Spiegel, A.M., Klee, W.A.: Development of opiate receptors and GTP-binding regulatory proteins in neonatal rat brain. J. Biol. Chem. 262: 8626-8630. 1987.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01031-19 LNC

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Conversion of Phenylalanine to Tyrosine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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	Michael Davis	Senior Staff Fellow	LNC NIMH
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	Yohsuke Minatogawa	Visiting Scientist	LNC NIMH
	Hans-Ulrich Siegmund	Visiting Fellow	LNC NIMH

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5.2

PROFESSIONAL:

4.2

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects
 (b) Human tissues
 (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Phenylalanine hydroxylase activity, measured at neutral pH, can be markedly increased by a brief exposure of the enzyme to alkaline pH.

Phenylalanine given to rats leads to an enhanced phosphorylation and activation of hepatic phenylalanine hydroxylase.

Major Findings:

During the course of our studies of the regulation of phenylalanine hydroxylase, we have found that a brief exposure of the enzyme to alkaline pH (pH 8.5-9.5) results in a marked (~10-fold) activation of the enzyme when it is assayed at neutral pH in the presence of its natural cofactor, tetrahydrobiopterin. These results indicate that on exposure to alkaline pH, the conformation of the enzyme changes to one that has higher catalytic activity and that this activated state persists for a time even when the enzyme is returned to neutral pH. We showed that this activated species of the enzyme does indeed have an altered conformation by demonstrating that the fluorescence of the enzyme changes when it is activated in this manner. We previously reported there is a similar correlation between activation of the enzyme and changes in its fluorescence spectrum during the course of activation by its substrate, phenylalanine.

A study of the mechanism of the alkaline pH activation of phenylalanine hydroxylase showed that it is a complex process. We have found that preincubation of the enzyme at alkaline pH primes the enzyme for substrate activation so that activation by phenylalanine of this primed species occurs at a much greater rate than that of the resting enzyme. But significantly, we have also shown that the primed species also has a two-fold greater intrinsic hydroxylase activity than the native enzyme. This latter finding represents the first example of an increase in the enzyme's intrinsic activity. It is significant because it disproves the currently widely-accepted generalization that asserts that all modes of activation of the hydroxylase simply reflect enhanced activation of the enzyme by its substrate.

We have also obtained evidence which indicates that not only are all modes of activation of the enzyme not simply reflections of substrate activation, but that even some examples of substrate activation are indirect and are the consequence of another kind of activation. It has been reported, e.g. that administration of phenylalanine to rats activates the enzyme. Although this activation has been attributed entirely to substrate activation, our results indicate that a significant part of this activation is due to phenylalanine-mediated phosphorylation of the hydroxylase.

Tetrahydrobiopterin, discovered in this laboratory approximately twenty-five years ago, is the physiological cofactor for the aromatic amino acid hydroxylases. Twenty years ago we showed that the auto-oxidation of this cofactor at neutral pH in phosphate buffer resulted in the formation of quinonoid dihydrobiopterin which rapidly rearranges to 7,8-dihydrobiopterin. (Only the quinonoid compound is a substrate for dihydropteridine reductase, the enzyme which regenerates tetrahydrobiopterin in vivo). Later it was shown that whereas quinonoid dihydrobiopterin also rearranges in acid to 7,8-dihydrobiopterin, under basic conditions 7,8-dihydropterin is formed i.e., the dihydroxypropyl side chain of biopterin is lost during the chemical rearrangement. More recently, an Australian group has reported, in apparent contradiction of our earlier finding, that at neutral pH quinonoid tetrahydrobiopterin is converted almost exclusively to 7,8-dihydropterin. Our reinvestigation of this chemical rearrangement has clarified the apparent

contradiction between our earlier results and those workers. Using three different methods for identifying the two putative products, (HPLC, multicomponent analysis of ultraviolet spectra, and chemical oxidation and analysis of the products), we have found that both pathways proceed but to differing extents depending on the reaction conditions i.e., the type of buffer, temperature, as well as the pH, all play a role in whether the side chain is lost during the chemical rearrangements. Under the conditions of our original experiments (neutral pH in phosphate buffer), our original results were replicated, i.e., the major ultimate product of BH_4 oxidation is indeed the corresponding 7,8-dihydrobiopterin. At higher pH values, the side-chain of biopterin does come off. Since the Australian workers studied different conditions than we did initially, they incorrectly concluded that the two sets of results were mutually exclusive. Our findings should help to delineate the conditions under which human tissues can be stored prior to analysis of tetrahydrobiopterin.

Significance to Biomedical Research and Proposed Course of Project:

Our new results on the regulation of phenylalanine hydroxylase have provided important insight into the relationship between activation of the enzyme by its substrate, phenylalanine, and other types of activation. The widely-accepted dogma asserts that all types of activation of the hydroxylase are merely different manifestations of substrate activation. According to this view, for example, activation by phosphorylation or activation by exposure to alkaline pH would simply prime the enzyme to be more rapidly activated by its substrate. Our results show, however, that with the latter type of activation, at least, part of the activation is due to an increase in the intrinsic activity of the hydroxylase that is quite independent of substrate activation, and part of it is substrate-mediated. These results, therefore, show for me first time that the accepted dogma is incorrect.

We have also shown that even some of the other accepted ideas about substrate activation must be modified. Our results indicate, for example, that an earlier published paper describing the direct in vivo activation of phenylalanine hydroxylase by phenylalanine is not due entirely to direct substrate activation. Rather, as we had previously postulated, part of the phenylalanine effect is due to a phenylalanine-mediated increase in phosphorylation of the enzyme.

These studies add support to the idea that substrate activation of phenylalanine hydroxylase is synergistically and reciprocally related to activation by phosphorylation, as well as to other forms of activation.

We plan to continue to explore the ways in which rat liver phenylalanine hydroxylase is regulated. We also plan to expand these studies to include the differential regulation of kidney phenylalanine hydroxylase using both biochemical and recombinant DNA techniques.

Publications:

1. Iwaki, M., Phillips, R. S. and Kaufman, S. Proteolytic modification of the amino-terminal and carboxyl-terminal regions of rat hepatic phenylalanine hydroxylase. J. Biol. Chem., 261: 2051-2056, 1986.
2. Rao, D. N. and Kaufman, S. Purification and state of activation of rat kidney phenylalanine hydroxylase. J. Biol. Chem., 261: 8866-8876, 1986.
3. Kaufman, S. Regulation of the activity of hepatic phenylalanine hydroxylase. In: Advances in Enzyme Regulation Weber, G., ed. Pergamon Press, Oxford, New York, Vol. 25: pp 37-64, 1986.
4. Davis, M., Kaufman, S. and Milstien, S. A modified ferrozine method for the measurement of enzyme-bound iron. J. Biochem & Biophys Meth., 13: 39-45, 1986.
5. Kaufman, S. Enzyme control by phosphorylation: Aromatic amino acid hydroxylases. In: The Enzymes, Boyer, P.D. and Krebs, E. G., eds, Academic Press, Orlando, Fl., Vol. 18: 218-281, 1986.
6. Kaufman, S. NADH dihydropteridine reductase from sheep liver. In: Methods in Enzymology, Colowick, S. P. and Kaplan, N. O., eds. Academic Press, Orlando, Fl, Vol. 142: pp 97-102, 1987.
7. Kaufman, S. The metabolic role of tetrahydrobiopterin. In: Chemistry and Biology of Pteridines, Cooper, B. A. and Whitehead, V. M., eds. Walter de Gruyter, Berlin, pp. 185-200, 1986.
8. Davis, M. D. and Kaufman, S. The effect of dietary iron on the activity of rat liver phenylalanine hydroxylase. In: Chemistry and Biology of Pteridines, Cooper, B. A. and Whitehead, V. M., eds. Walter de Gruyter, Berlin, pp. 363-367, 1986.
9. Parniak, M. A., and Kaufman, S. Alterations in cofactor-dependent activity of phenylalanine hydroxylase as a function of pH. In: Chemistry and Biology of Pteridines, Cooper, B. A. and Whitehead, V. M., eds. Walter de Gruyter, Berlin, pp. 355-358, 1986.
10. Kaufman, S. The enzymology of the aromatic amino acid hydroxylases. In: Amino Acids in Health and Disease: New Perspectives, Kaufman, S., ed. UCLA Symposia on Molecular and Cellular Biology held at Keystone, CO., May 30-June 4, 1986. Alan R. Liss, Inc., New York, pp. 205-23332, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01032-19 LNC

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthesis of Catecholamines

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	Seymour Kaufman	Chief	LNC NIMH
	Thomas Nelson	Staff Fellow	LNC NIMH
	Dominique Pigeon	Visiting Fellow	LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.2

PROFESSIONAL:

1.2

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

A new rapid isolation procedure has been developed for the purification of tyrosine hydroxylase from brain and adrenal tissue.

A phosphatase that catalyzes the dephosphorylation-deactivation of tyrosine hydroxylase has been partially purified from rat brain. Its activity is affected by the pterin coenzyme for tyrosine hydroxylase.

Project Description:

We have continued our studies of brain and adrenal tyrosine hydroxylase using our new rapid, high-yield purification procedure. We have also devised several new methods for determining protein-bound phosphate (Pi) that are far more sensitive than previously published procedures. Using the new hydroxylase purification procedure in conjunction with these very sensitive Pi assays, we have determined, for the first time, the amount of protein-bound Pi in both pure brain and adrenal tyrosine hydroxylases. Since the enzyme was isolated in the presence of phosphatase inhibitors, the values of protein-bound Pi, about 0.07 moles/mol of hydroxylase subunit, probably reflect the Pi content of the enzyme in the resting state. With this value for the endogenous Pi content, together with a determination of the change in hydroxylase activity as a function of 32 Pi incorporated (mediated by cAMP-dependent protein kinase), we were able to obtain, for the first time, a detailed picture of the way hydroxylase activity varies with the total content of protein-bound Pi. With the brain enzyme, hydroxylase activity is not a linear function of Pi content, a result that suggests a small allosteric effect.

We have also partially purified from rat brain a phosphatase that catalyzes the dephosphorylation (and deactivation) of phosphorylated tyrosine hydroxylase. This phosphatase appears to be regulated in opposite ways by GTP, the precursor of BH_4 , and by BH_4 itself; the former compound inhibits, whereas the latter compound stimulates the phosphatase.

Significance to Biomedical Research and Proposed Course of Project:

The characterization of a phosphatase that catalyzes the dephosphorylation-mediated deactivation of tyrosine hydroxylase should enable us to obtain a much more complete picture of how the state of phosphorylation of tyrosine hydroxylase affects its activity. The demonstration that the phosphatase is inhibited by GTP and activated by BH_4 suggests a heretofore unsuspected way in which the coenzyme for the hydroxylase, BH_4 , can affect the activity of the hydroxylase. This observation has already suggested new interpretations for some puzzling old data in the literature.

We plan to continue to characterize this phosphatase and to study its interaction with tyrosine hydroxylase.

Publications:

1. Nelson, T. and Kaufman, S. Two enzymatic methods for determination of the phosphate content of phosphoproteins. Anal. Biochem. 161: 352-357, 1987.
2. Nelson, T. and Kaufman, S. Interaction of tyrosine hydroxylase with ribonucleic acid and purification with DNA-cellulose or poly (A)-sepharose. Archives of Biochem and Biophys 257: 1987.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01038-19 LNC

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phenylketonuria and Other Diseases Caused by Defects in Biopterin-Dependent Enzymes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI	Seymour Kaufman	Chief	LNC NIMH
	Sheldon Milstien	Research Chemist	LNC NIMH
	Stanley Rapoport	Chief	LN NIA

COOPERATING UNITS (if any)

Lab. of Neurosciences, National Institute on Aging

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

TOTAL MAN-YEARS:

0.7

PROFESSIONAL:

0.7

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

A determination of neopterin and biopterin levels in amniotic fluid has been used in the prenatal diagnosis of a possible case of a defect in tetrahydrobiopterin synthesis.

Alzheimer patients have low CSF levels of tetrahydrobiopterin.

Project Description:

The goal of this research project is the detailed description, at the molecular level, of diseases caused by defects in components of the aromatic amino acid hydroxylating systems.

Major Findings:

We have successfully carried out one of the first prenatal diagnoses of a defect in the de novo synthesis of tetrahydrobiopterin. The method used was based on our previous demonstration (Nixon et al. J. Neurochem. 35: 898-904, 1980) that this disease can be detected by a determination in urine of neopterin (N) and biopterin (B), patients with the disease having an elevated N/B ratio. We reasoned that the same metabolic abnormality would be found in amniotic fluid. In this case, the N/B ratio indicated that the fetus (17 to 18 weeks of gestation) was not homozygous for the trait and advised that the pregnancy be carried to term. The baby was normal at birth and continues to show no signs of the disease. There has been only one other example of prenatal diagnosis of this condition carried out by a Swiss group, which independently utilized the same concept and similar methodology.

We, as well as others, have found that patients with Alzheimer's disease have a significant decrease in the concentration of BH_4 in their cerebrospinal fluid. Direct measurements of the activities of the BH_4 biosynthetic enzymes in the cortex of a single case of senile dementia of the Alzheimer type have so far not detected any decreases compared to one control.

Significance to Biomedical Research and Proposed Course of Project:

Our findings that patients with Alzheimer's disease have decreased concentrations of BH_4 in their CSF but not in their blood has required a major revision of the idea that these patients suffer from a generalized deficiency of BH_4 . Further studies are being carried out to determine whether it will be possible to identify the cause of the decreased BH_4 levels in this disease. We also plan to explore the question of whether a deficiency of BH_4 is a consequence or a cause of the disease.

We plan to try to isolate cDNA clones to the four enzymes involved in BH_4 synthesis and to use these clones for prenatal diagnosis of variant forms of phenylketonuria caused by defects in these enzymes.

Publications:

1. Kaufman, S. Tetrahydrobiopterin and hydroxylation systems in health and disease. Neurochem & Neuropharmacol, 142: 1-28, 1986.
2. Kaufman, S. Unsolved problems in the diagnosis and therapy of hyperphenylalaninemia caused by defects in tetrahydrobiopterin metabolism. J. Pediatr 109: 572-578, 1986.

3. Irons, M., Levy, H. L., O'Flynn, M. E., Stack, C. V., Langlais, P. J., Butler, I. J., Milstien, S. and Kaufman, S. Folinic acid therapy in the treatment of dihydropteridine reductase deficiency. J. Pediatr, 110: 61-67, 1987.
4. Kaufman, S. Classical phenylketonuria and its variants caused by defects in bipterin metabolism. In: Amino Acids in Health and Disease: New Perspectives, Kaufman, S., ed. UCLA Symposia on Molecular and Cellular Biology held at Keystone, CO., May 30-June 4, 1986. Alan R. Liss, Inc., New York, p. 517-538, 1987.
5. Kay, A. D., Milstien, S., Kaufman, S., Creasy, H., Hoxby, J. V., Cutler, N. R. and Rapoport, S. Cerebrospinal fluid bipterin is decreased in Alzheimer's disease. Archives of Neurol. 43: 996-999, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01039-19 LNC

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pteridine Biosynthesis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI Sheldon Milstien	Research Chemist	LNC NIMH
Seymour Kaufman	Chief	LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION
ADAMHA, NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	

CHECK APPROPRIATE BOX(ES)

(a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

A scheme has been developed to isolate and characterize all of the enzymes of tetrahydrobiopterin (BH₄) biosynthesis from rat brain. The wide distribution of tetrahydrobiopterin and these enzymes in the brain as well as the lack of effect of specific neurotoxins on some of the enzymes in the pathway suggests that tetrahydrobiopterin and/or these biosynthetic enzymes may have other roles than previously thought. Work is now under way to prepare antibodies to each of these enzymes to use for molecular cloning to obtain DNA probes to investigate the physiological roles.

Project Description:

It is now clear that the availability of tetrahydrobiopterin, the cofactor required for the hydroxylation of tyrosine and tryptophan, can be a determining factor in the synthesis of those neurotransmitters which are derived from the hydroxylated amino acids. Thus, any physiological condition which alters the metabolism of tetrahydrobiopterin could have profound CNS effects. In order to better understand the mechanisms by which tetrahydrobiopterin levels are regulated as well as investigating its role in the pathophysiology of such disorders as Alzheimer's disease and variant forms of PKU, we are isolating the enzymes which catalyze the *de novo* biosynthesis of tetrahydrobiopterin. Antibodies are being prepared to all of the proteins which can then be used for characterization of the proteins in human tissues as well as to screen genomic libraries to prepare cDNAs which will be very valuable in genetic screening.

Major Findings:

The four enzymes which catalyze the *de novo* biosynthesis of BH_4 from GTP, GTP-cyclohydrolase, 6-PPH₄ synthase, 6-PPH₄ reductase, and sepiapterin reductase have all been isolated and characterized from rat brain. The enzymes, as well as BH_4 itself, are fairly evenly distributed in the rat brain. There is a good correlation between the concentration of BH_4 and the activity of GTP-cyclohydrolase and the activities of tryptophan and tyrosine hydroxylases. However, treatment of rats with specific nigro-striatal toxins causes a concomitant decrease in BH_4 , tyrosine hydroxylase and GTP-cyclohydrolase without having any significant effect on 6-PPH₄ synthase, 6-PPH₄ reductase, or sepiapterin reductase. These results suggest either that the terminal enzymes in the BH_4 biosynthetic pathway are located in other neurons or that only a small fraction of these enzymes is co-localized with tyrosine hydroxylase and that the major portion of these enzymes located in other neurons must have other functions.

Significance to Biomedical Research and Proposed Course of Project:

The characterization of the four enzymes involved in the *de novo* synthesis of BH_4 will help to precisely localize the enzyme defects in this pathway that can lead to abnormal brain development and function. We plan to continue to try to prepare antibodies to all of the enzymes. Such antibodies will be used in attempts to isolate cDNA clones for each of the enzymes.

Publications:

1. Milstien, S. and Kaufman, S. The biosynthesis of tetrahydrobiopterin in rat brain. In: Chemistry and Biology of Pteridines, Cooper, B. A. and Whitehead, V. M., Walter de Gruyter, Berlin, pp. 169-181, 1986.

2. Milstien, S. and Kaufman, S. The oxidation of apomorphine and other catechol compounds by horseradish peroxidase. Relevance to the measurement of dihydropteridine reductase activity. Biochim. Biophys. Acta, 923:333-338, 1986.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01040-19 LNC

PERIOD COVERED

October 1, 1986 through September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Biology of the Pterin-Dependent Hydroxylases and Ancillary Enzymes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI Seymour Kaufman	Chief	LNC NIMH
Sheldon Milstien	Research Chemist	LNC NIMH
Bruce Citron	Senior Staff Fellow	LNC NIMH
Y. C. Liu	Visiting Fellow	LNC NIMH
John Donlon	Visiting Scientist	LNC NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.2

PROFESSIONAL:

2.2

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A sufficiently extensive cDNA clone of rat liver phenylalanine hydroxylase has been isolated and sequenced.

A composite DNA molecule containing this cDNA and the E. coli lac promoter, operator, and translation initiation site has been constructed and placed in a host that is inducible for this operon.

Elevated phenylalanine hydroxylase mRNA levels have been observed in diabetic rats.

Project Description:

Through a combined, interdisciplinary approach, we are studying the structure-function relationships of the aromatic amino acid hydroxylases and related enzymes to explain the precise biochemical mechanisms involved in the reactions which these enzymes catalyze. Importantly, with the aid of molecular biology, we can more readily modify these enzymes in very specific ways to clearly define the molecular interactions required for proper catalysis. We also plan to use cDNA clones of these enzymes as probes to study aspects of the molecular genetics of certain neurological and psychiatric disorders.

Major Findings:

Phenylalanine Hydroxylase

We have isolated many cDNA clones to rat phenylalanine hydroxylase and several of these have been sequenced to identify a few partial N-terminal and C-terminal cDNA inserts and one nearly full length cDNA isolate. The full length cDNA contains a 1916 base-pair insert which includes the coding sequence from asparagine 8 to the C-terminal serine 452 and continues to the poly(A) sequence 568 nucleotides further downstream.

Studies of hormonal regulation of phenylalanine hydroxylase activity in diabetic rats have indicated that a three fold increase of enzyme activity in the liver seems to be correlated with a five-fold increase of phenylalanine hydroxylase mRNA.

Tyrosine Hydroxylase and Tryptophan Hydroxylase

Several putative cDNA clones have been isolated from the human brain stem and also the basal ganglion. These have been partially characterized by restriction mapping and southern hybridization.

Dihydropteridine Reductase

An intact DNA fragment has been isolated from the full length cDNA clone, which contains only the dihydropteridine reductase gene.

6-pyruvoyl-tetrahydropterin synthase

Antibody specific for this protein has been produced and shown to identify the enzyme band on a Western blot.

Significance to Biomedical Research and Proposed Course:

Phenylalanine Hydroxylase

Phenylketonuria results from an untreated deficiency of phenylalanine hydroxylase. A major symptom of this disease is severe, irreversible mental retardation. This indicates that individuals carrying a mutant allele that

yields a partially deficient enzyme should have some propensity for mental disorders. We will be using cDNA probes to characterize alterations in messenger RNA levels that have biological effects.

Tyrosine Hydroxylase

This enzyme is the primary control point for the neurotransmitter pathway implicated in several diseases such as Parkinsonism. Recent findings have been consistent with at least one type of mutation in this gene being responsible for at least one type of manic depressive disorder. Using cDNA probes, we will study the transcriptional response of a wild-type copy of this gene in neuroblastoma cells to a wide variety of stimuli. These probes will also be used to characterize the modifications in tyrosine hydroxylase that lead to some of the diseases explainable by altered catecholamine metabolism.

Tryptophan Hydroxylase

The neurotransmitter, serotonin, is an end product of the metabolism of tryptophan by tryptophan hydroxylase. Decreased activities of all three aromatic amino acid hydroxylases produce pronounced central nervous system disorders broader than the symptoms due to any characterized or postulated abnormality of any one hydroxylase. Conversely, there is a large array of mental diseases for which no biological explanation exists. Aberrant tryptophan hydroxylase expression might be responsible for some of these disease states and we will attempt to identify these cases using hybridization probes.

Dihydropteridine Reductase (DHPR) and 6-pyruvoyl-tetrahydropterin synthase (PTS).

All three aromatic amino acid hydroxylases have an absolute requirement for the cofactor, tetrahydrobiopterin. DHPR and PTS are involved in the production of this cofactor and individuals deficient in one of these activities have atypical phenylketonuria. This is a severe disease involving mental retardation, CNS problems, movement disorders, etc. Several patients have been identified having marked deficiencies in each of these enzymes. We will first characterize the genetic basis in the known cases and also use these probes to identify changes in less severe and less obvious classes of such disorders.

Publications:

1. Citron, B. A., Chaudary, P. V., Rao, D. N. and Kaufman, S. Evidence for transcription and potential translation of the human 1.9 kb HindIII repetitive element. Nucleic Acids Res, 14: 3137-3142, 1986.
2. Lockyer, J., Cook, R. G., Milstien, S., Kaufman, S., Woo, S. L. C. and Ledley, F. D. Structure and expression of human dihydropteridine reductase. Proceed. Natl Acad Sci, 84: 3329-3333, 1987.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00981-21 LNP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanical, Thermal and Optical Signs of Excitation in the Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Ichiji Tasaki Chief, Unit of Neurobiology LNP, NIMH

Others: Nobuko Tasaki Guest Worker LNP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Unit on Neurobiology, Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Due to the transfer of principal investigator to the Laboratory of Cell Biology, this project has been assigned number Z01 MH 02396-01 LCB.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01092-09 LNP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less Title must fit on one line between the borders.)

The Frontal Lobe and the Cerebral Control of Behavior

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Steven P. Wise	Research Biologist	LNP, NIMH
Others:	Kiyoshi Kurata	Visiting Fellow	LNP, NIMH
	Eilon Vaadia	Visiting Associate	LNP, NIMH
	Shraga Hocherman	Visiting Associate	LNP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Animal Center, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

2.4

PROFESSIONAL:

2.4

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects
 (b) Human tissues
 (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The primate frontal lobe consists of three main parts: the primary motor cortex (MI), the prefrontal cortex, and the nonprimary motor cortex. Previous work on this project has shown that the nonprimary motor cortex can be divided into at least two fields: the supplementary motor cortex (SM) and the premotor cortex (PM). Definition of these cortical fields depended on an analysis of neuronal responses to peripheral inputs, thresholds for evoking movements with intracortical electrical stimulation, the properties of single neurons during the performance of several visuomotor tasks, and cytoarchitectonics. After defining the areas, we directed our studies, using the techniques of behavioral neurophysiology developed in this laboratory, toward the nonprimary motor cortex. Our results support the hypothesis that PM and SM contribute to sensorially-referenced behaviors especially those guided by abstract or arbitrary sensory cues. These studies have provided new insight into the processes by which animals, including humans, prepare for action in relation to partially predictable environmental events. The preparation for receipt of informa(attention) and for future action (motor set) represent higher brain functions amenable to both quantitative and qualitative neurophysiological analysis and appear to be a property conferred by the frontal cortex.

Objectives:

The goal of this project is to gain a better understanding of functional organization of the primate frontal lobe and to contribute to the development of a unified hypothesis of its role in directing behavior. In order to understand the biological basis of mental illness, it is necessary to have a better understanding of the processing underlying the behavioral flexibility of higher mammals --- i.e., the ability to respond with virtually any motor action of which the animal is capable to virtually any sensory input that the animal can discriminate, to plan for future action, and to formulate and achieve reasonable goals. We believe that the mechanisms of the frontal cortex underlie these important higher brain functions. Our approach to an analysis of frontal lobe mechanisms consists of a multidisciplinary experimental strategy involving the formulation and testing of hypotheses with the techniques of behavioral neurophysiology developed in this laboratory.

Methods:

Fourteen rhesus monkeys have been trained to perform visually guided motor tasks over the nine-year course of this project. Each one of the eight tasks described below is a separate subproject:

(1) One monkey was operantly conditioned to depress one of four keys located in a perimeter at arms length. While the monkey pressed one key, another of the four keys, selected randomly, was illuminated after a randomly varied delay period. This key thereby became the next target. An auditory cue near the target could be substituted for illumination of the target. A barely discernable visual cue near the target key, appearing after another variable delay, signaled the monkey to move and depress the target. The monkey was required to make the movement within a short period of time, near the limit of reaction time. The purpose of this subproject was to make an initial survey of neuronal activity patterns in PM to help define it. Electrical stimulation and cytoarchitectonic techniques were used in this subproject. Another important aspect of this subproject was a comparison of neuronal activity when visual vs. auditory signals instruct the monkey to make the same movement.

(2) Two monkeys were conditioned to align two spots of light on a screen. One of these spots was controlled by a computer (the target spot), the other by the arm movements of the animal (the position spot). The monkey was required to align the spots within a small accuracy "window." In five-sixths of the trials, after a short period of time, the target spot jumped to one of six locations. The monkey had to maintain his arm position unchanged until the target spot dimmed, at which point it was required that the monkey flex or extend his forearm rapidly and accurately in order to realign the position spot with the target spot. In one-sixth of the trials, the computer selected a situation in which physically identical stimuli signaled the animal to make no movement. This experiment was designed for two purposes: to contrast neuronal activity in MI and PM, and to distinguish neuronal activity when identical stimuli signal the execution vs. the withholding of movement.

(3) Two monkeys were operantly conditioned to depress the central of three keys located on a panel at arm's reach. After a period of time, either the left or right key became illuminated. Three experimental conditions ensued: (a) the left or right key remained illuminated and served as the target for the subsequently triggered movement, (b) the light was turned off before the monkey was allowed to execute the movement, forcing the monkey to remember the proper target, or (c) the target light was switched before the monkey was allowed to execute the movement. This experiment was designed to further test the relationship of neurons in PM to the motor set of the animal. In addition, we could compare cell activity when the monkey performed the task described above and a self-paced movement between the left and right keys. Subproject 3 was designed to test the competing hypotheses that set-related activity in PM reflects a continued visual stimulus and that it reflects the preparation for movement.

(4) One monkey was conditioned to execute a single limb movement as well as a short sequence of two limb movements in the same direction. The monkey was seated in front of a panel of three keys as in subproject 3. Each trial started with the monkey pressing the leftmost of the three keys. Two experimental conditions ensued: (a) the center key was illuminated, thus indicating that a single movement was to be made to depress the center key, or (b) both the center and right lights were simultaneously illuminated to indicate that a short motor sequence was to be initiated to depress both keys, in order. This subproject was designed to test the hypothesis that PM is especially important in guiding simple sequences of movement.

(5) Two monkeys were conditioned to respond to two different sorts of visuospatial instruction signals. One type of instruction signal was comparable to that described in the subprojects described above, i.e., the visual cue itself was directional; indeed it was part of the target. This situation could be contrasted with one in which the instruction cues contained no directional information. A blue lamp meant to move the limb to the right and a yellow lamp to move to the left. Thus the relationship of the stimulus to the movement in the latter situation was abstract or arbitrary. The hypothesis was that if PM activity reflects the preparation for movements there should be little or no difference in activity in the two situations.

(6) Two monkeys were conditioned to make the same movements under two different experimental conditions: (a) when visual instructions of the type described for subprojects 1, 3 and 5 guided the movement, and (b) when the identical cues were irrelevant or nonexistent and the monkey guided its behavior via internal (i.e., nonsensory) processes. This study was designed to test the hypothesis that PM is especially important when sensory signals instruct a movement and to contrast activity in PM with that in SM, which has been hypothesized to play a special role when internal processes instruct a movement (P.E. Roland et al, J. Neurophysiol., 1980, 43: 118; J.C. Eccles, Arch. Psychiat. Nervenkr., 1982, 231: 423; G. Goldberg, Behav. Brain Sci., 1985, 8: 567).

(7) Two monkeys were trained to respond to one visual stimulus with a hand movement and to another visual stimulus with a foot movement. The cell activity in PM before hand movements was compared to that before foot movements. Before

either movement, the monkey was required to withhold movement for a variable delay period. This study was designed to examine the internal organization of the premotor cortex, in particular whether it was topographic organization.

(8) The activity of cells in PM of one monkey was compared in two experimental conditions: (a) when a visual cue instructed the monkey about where the target of the next limb movement should be, as well as when to execute the movement, and (b) when a visual cue indicates to the monkey only when to execute the movement. In condition b, the monkey had no environmental cue upon which to base its choice of two potential targets. Instead, the monkey simply had to guess and was prevented from adopting the strategy of moving always to one target (thereby receiving rewards on half the trials) by an algorithm that randomly selected what target would be rewarded. That algorithm decreased the probability that a movement to a given target would be rewarded by 0.1 every time the monkey made as many as three consecutive movements to that target. This study was designed to examine the neuronal activity in PM that precedes the instructional cues, in particular to test the hypothesis that such activity reflects the preparation for movement.

In each of the subprojects, single-unit activity and behavioral data were collected on-line with PDP 11/03 and 11/23 computers and analyzed off-line with PDP 11/23 and 11/73 computers. Presently, we are analyzing the data with a dedicated PDP 11/73 in the laboratory, using a program written by Karl Arrington, formerly of the LNP support staff.

Major Findings:

The activity of about 3600 neurons have been examined in this project to date, and 1583 of these have been studied in detail. Three major sets of findings developed from our work on this project and three others have spun off to other projects: Z01 MH 01097-01 LNP headed by Melvyn Heyes; Z01 MH 01096-03 LNP headed by Andrew Mitz; and Z01 MH 02376-01 LCB headed by Charles Gerfen.

1. Comparison of Primary and Nonprimary Motor Cortex. SM neurons were found to be much less responsive to peripheral somatosensory inputs than MI neurons in the same monkeys. The lack of profound somatic sensory responsiveness in these parts of the somatic sensorimotor cortex supports the hypothesis that SM plays its most significant roles in the guidance of movement by internal processes (such as memory) rather than by feedback from mechanoreceptors of the limbs. We have begun an explicit test of this idea (subproject 6). MI seems to be specialized for control of movement, in part, by cutaneous and noncutaneous mechanoreceptors, and adjustment for internal or external events that cause deviations from the animal's goals.

2. Cortical Field Definition and Internal Organization of Nonprimary Motor Cortex. Our findings have enabled us to improve the current understanding of cerebral localization and functional specialization in the agranular frontal cortex (SM, PM, plus MI) and certain adjacent parts of the cortex. Of special importance has been the effort to determine anatomical correlates of physiologically defined cortical regions. Microelectrode methods revealed that the

boundary between MI and SM corresponds to the boundary between two anatomically defined parts of the agranular frontal cortex (termed areas 4 and 6). This differed from the accepted published maps at the time this study was undertaken. This line of inquiry has spun off to Mitz's project, as mentioned above. Similar work has clarified the location of the boundary between PM and MI, and this work, too, is being elaborated as part of Mitz's project. In this fiscal year, we found in this project that most PM neurons contribute to the preparation for and execution of specific limb movements rather than movement per se and thus has a coherent internal organization. Further, the differential distribution of neurons with activity related to hindlimb vs. forelimb movement (subproject 7) support previous indication that PM is topographically organized (K. F. Muakkassa, and P. L. Strick, Brain Res., 1979, 177: 176; K. Kurata, K. Okano and J. Tanji, Exp. Brain Res., 1985, 60: 188).

3. Premotor Cortex Physiology. The findings described above led to the elaboration of a related, third set of findings, those concerning the functional specializations of PM and SM. PM can be distinguished from the MI representation by its markedly increased threshold for evoking movements with intracortical microstimulation. Further, and of most interest to us, a substantial population of neurons change their activity in relation to motor set. The pattern of activity termed "set-related" appears to be specifically correlated with the motor preparation of the animal (subproject 1). This hypothesis has been supported in six ways: (a) set-related units show changes in activity when visual signals cue a movement, thus establishing a specific motor set, but not when the same signals instruct the monkey to withhold movement (subproject 2); (b) if the visual instruction changes (to establish a different motor set), the unit activity rapidly changes to reflect the new set (subproject 3); (c) when the instruction is removed (but the set remains the same), the unit activity continues to reflect the set rather than the sensory signals (subproject 3); (d) the set-related activity before the first of a series of two movement is the same as that before the same movement when it is executed by itself (subproject 4); (e) set-related activity is usually the same when directional (left or right) a instruction stimulus and an arbitrary (yellow or blue) instruction stimulus instruct the same movement (subproject 5); and (f) set-related activity is usually the same when the monkey plans a movement on the basis of visual (external) stimuli as when the monkey plans the same movement on the basis of mnemonic (internal) information (subprojects 6 and 8). In addition, it has been found that these and other premotor cortex units change their activity in advance of predictable environmental events, and that such activity which we call pre-cue activity, reflects or contributes to attention toward the instructional information transmitted by the cue, as well as its timing (subprojects 2, 3, 6 and 8). Taken together our findings improve the understanding of the set-related processes of PM and accord with the hypothesis that PM plays an important role in behaviors in which a movement must be retrieved from memory on the basis of highly flexible, arbitrary cues.

The past year has seen the completion of data collection on subprojects 6, 7, and 8. We had accepted for publication a full-length paper on subproject 5 in Experimental Brain Research. In addition, this fiscal year saw the publication of results from subprojects 2 and 3 (Wise et al. 1986) and a complete review of the literature on the motor cortical fields of rodents, a basis for comparison

with the primate research performed in the present project (Wise and Donoghue, 1986). Toward the end of the fiscal year, we submitted certain of the results of subproject 6 for publication and we are currently preparing full-length manuscripts based on the results of subprojects 7 and 8. As of this writing, we hope that these papers will be submitted for publication before the end of the fiscal year. In addition, at least one and possibly more papers are expected to result from these data in the next fiscal year.

Significance to Biomedical Research and to the Program of the Institute:

Studies of functional localization in higher-order motor cortical fields, such as the premotor cortex and supplementary motor cortex, are important to understanding the cortical control of motor acts of the least automatic kind, in both health and disease, and especially for understanding the way in which sensory signals are converted, by the brain, into organized motor acts. A much improved knowledge of the nonprimary areas of the cerebral cortex and their relation to higher-order control of motor behavior may yield insight into higher brain functions of all types. More generally, much of this project is devoted to basic study of the frontal cortex, a presumed site of higher brain functions such as reason, attention, forethought, and perception, as well as fine motor capabilities. In this context, the recent findings of Weinberger and his group at St. Elizabeth's Hospital are of great interest. They have hypothesized that disease of the frontal lobe is one cause of schizophrenia (D.R. Weinberger, K.F. Berman, and R.F. Zec. Archiv. Gen. Psychiatry, 1986, 43: 114-124). We hope to link our growing understanding of the frontal lobe mechanisms underlying "motor set", the ability of animals to plan and select an action in advance of the time that it needs to be performed, with the activity of frontal cortex neurons. Such advances would be of fundamental importance in understanding the roles of the frontal lobe in mental health and disease.

Proposed Course of the Project:

The project is being elaborated in two directions: (1) an explicit examination of the functional organization of the prefrontal cortex (PF) in collaboration with Eilon Vaadia and Shraga Hoeherman) and (2) a continuation of our study of the premotor cortex (PM) and its role in the selection and control of behavior.

1. A monkey will be operantly conditioned to perform a visually guided behavior involving three features: (a) if two signals, presented sequentially, are the same (i.e., red plus red or green plus green) then, after a self-timed delay period, the monkey is required to displace the target key displaying that color cue (red or green, respectively); (b) if the two signals are different (i.e., one is red and the other green), then the monkey must either withhold any limb movement. This task is directed toward further testing the hypothesis that PM neuronal activity reflects the preparation for action (e.g., S.P. Wise et al., Brain Res., 1983, 260: 301) whereas PF functions in comparing incoming information against memories and in the application of behavioral rules.

This new subproject will extend our work on PM into the PF cortex and to explore directly (and in the same animal) differences in activity in these two cortical regions. In the past fiscal year, we have trained a monkey to perform this task

and have begun the data collection. Vaadia and Wise continued data collection until Vaadia's departure in August, at which time Hocherman began his work on this subproject. We expect the first phase of this work to be completed next fiscal year.

2. The behavioral neurophysiological studies of PM have many more planned sub-projects than time and manpower to perform them. Accordingly, one important line of investigation was spun off to Mitz's project (Z01 MH 01096-03): an examination of frontal lobe function in relation to memory. The only past studies of memory-related neuronal activity in frontal cortex have either been indirect, equivocal or based on complex and poorly localized summed potentials rather than the activity of individual neurons. Accordingly, and in view of the recent proposal by U. Halsband and R.E. Passingham (Behav. Brain Res., 1985, 18: 269) that PM functions in retrieving a movement from a long-term memory store, we propose to test this hypothesis by examining PM activity both before and after learning an arbitrary stimulus-movement association. We predict that there will be little or no task related activity following the stimulus until the association is learned.

We are planning two initiatives in the present project. In one, we plan to examine frontal cortex neuronal activity to determine the frontal cortical location of neurons subserving sensory memories and the locations of analogous representations of motor memories. The experimental design to achieve our objective can be summarized: the monkey must learn to associate a color (there must be two) in a certain location in space (there must be four) with a specific motor act (there must be two). In one condition, the monkey is compelled to remember the sensory stimulus to be able, after a delay period, to execute the correct behavior. In the other condition, the monkey must remember the motor act associated with the visuospatial stimulus. It will be time consuming to condition monkeys to perform this difficult task, but we believe that it will be a very rewarding study if successfully completed. In terms of cerebral localization, the distinction being tested, that between the sensory and the motor, between input and output, between perception and action, between memories and the behavioral uses of those memories, is one of the most important problems in neurobiology, one which is best approachable with the tools of behavioral neurophysiology.

Publications:

Kurata, K and Wise, S.P.: Premotor cortex of rhesus monkeys: Set-related activity during two conditional motor tasks. Experimental Brain Res. in press, 1987.

Wise, S.P.: Neuroanatomical substrates of premotor centers. Progress in Brain Research 64: 111-114, 1986.

Wise, S.P.: The motor cortex. In Adelman, G. (Ed): The Encyclopedia of Neuroscience, Springer-Verlag, in press, 1987.

Wise, S.P. and Donoghue, J.P.: The premotor cortex of rodents. In Peters, A. and Jones, E.G. (Eds.): Cerebral Cortex, Vol 5, Plenum, New York, 1986, pp. 243-270.

Wise, S.P., Weinrich, M., and Mauritz, K.-H.: Movement-related activity in the premotor cortex of rhesus macaques. Progress in Brain Research, 64: 117-131, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01096-03 LNP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Spatial Organization of the Primate Motor Cortex

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Andrew R. Mitz	Senior Staff Fellow	LNP, NIMH
Others:	Steven P. Wise	Research Biologist	LNP, NIMH
	Moshe Godschalk	Visiting Associate	LNP, NIMH

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TOTAL MAN-YEARS:

1.9

PROFESSIONAL:

1.9

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to examine the internal organizations and the inter-relationship among motor areas of the frontal lobe, including the primary motor cortex (MI), the supplementary motor cortex (SM), the frontal eye fields (FEF) and the premotor cortex (PM). The model species chosen for study is the rhesus monkey, because these motor areas have been best characterized in this species. In the first part of this project intracortical microstimulation is being employed to examine the efferent topography of the motor areas.

In the second part of the project single-unit recording will be employed to examine the possible involvement of the premotor cortex in learning new stimulus-response relationships. It is the goal of this part of the project to determine the roles played by each cortical motor area in recall and execution of learned motor behaviors.

Objectives:

Movement-related single-unit activity in the primary motor area (MI), supplementary motor area (SM), premotor area (PM), and frontal eye field (FEF) suggest that these cortical regions are involved in the programming and execution of movement. Anatomical evidence further suggests that these areas are extensively interconnected, and we hypothesize that these cortical fields act together to generate appropriate motor commands. Although certain features of the anatomical and physiological organization of these motor areas are known, many questions remain about the detailed topography in each of these areas. The objective of this project is to explore the topographic organization of these motor areas and correlate their functional organization with aspects of their cytoarchitectonics. A further goal is to characterize and distinguish each area's contribution to descending motor commands.

Methods Employed:

1. Microstimulation mapping of cortical motor areas. Intracortical microstimulation in awake or lightly sedated animals has become a standard method for examining motor topography in MI, FEF, and other motor areas. However, this technique had not been successful for exploring the somatotopy of SM or PM.

The problem of SM mapping has been overcome in this project by developing a modified intracortical stimulation technique. This technique utilizes platinum-iridium electrodes with about 1000 μm^2 of metal exposed at the tip, compared to standard microelectrodes with exposed tips of 100-250 μm^2 . In addition to the electrode geometry, careful selection of stimulus parameters and the use of biphasic current pulses has contributed to successful SM mapping.

Rhesus monkeys were implanted with stainless steel chambers to expose the dura mater over SM, PM, and FEF. Electrodes were inserted through the dura and electrical stimuli were delivered periodically as the electrode descended through the cortex. The stimulus parameters were 0.2 ms duration for each phase of the biphasic pulses, 330 pulses/second, 31-pulse trains, and 65 μa search currents. Movements evoked at each stimulation site were identified by two observers and recorded.

2. Oculometry. In some instances eye movements were observed and recorded manually. Later, eye movements were recorded as electro-oculograms via implanted silver/silver-chloride electrodes or non-invasively with infrared oculometry. When the latter techniques were used, two channel directional information (left-right and up-down) was either recorded on separate magnetic tape channels or combined as an X-Y vector on a storage oscilloscope and photographed.

3. Quantitative cytoarchitectonics. The boundary between cytoarchitectonic areas 4 and 6 was first estimated qualitatively. Then, a computerized cell plotting system connected to a high-power microscope was used to locate, identify, and measure individual cells on a series of histological sections. Cell body areas were measured in 21 sections separated by about 500 μm , and covering a 10 mm rostrocaudal extent. Cell measurements were made from an area 1 mm in dorsoventral extent and centered midway between the dorsal surface of the hemisphere and the cingulate sulcus. Each cell body greater than 20 μm in any dimension was

circumscribed under 400 X magnification. The coordinates of the cell boundary were recorded by the computer, which then computed the area of the cell body. Any stained portion of the proximal dendrites was included in the cell area measurement.

4. Chronic single-unit recordings. Movement-related units in the motor areas were isolated using chronic recording techniques in an operantly conditioned monkey. The behavioral paradigm chosen dissociated the direction of hand displacement from the direction of wrist rotation by training the animal to make wrist flexion/extension movements from either a pronated or supinated posture. When the forearm was pronated, wrist flexion was a downward movement of the hand; when the forearm was supinated, wrist flexion was an upward movement. Isolated units from MI were tested in both forearm positions. Paradigm events, wrist position, and unit firing times were recorded in real-time using a Plessey Micro II (LSI 11/23) minicomputer. After each recording session, perievent spike histograms were generated from the data to compare the event-related activities of individual task-related neurons.

A new behavioral paradigm is being used to evaluate PM unit activity before and after stimulus-response associations are learned. New hypotheses concerning the functions of the PM suggest that one of its roles is to recall an appropriate motor program based upon recognition of a stimulus. To study the validity of this approach, monkeys will be trained to move a lever to one of 3 positions based upon 3 color figures. The set of color figures will be changed periodically to restart the learning process. Single-units will be studied as the animal learns which figure matches which target position. Unit activity will also be recorded during performance of a well-learned stimulus-response association. The hypothesis will be supported if the response of a PM unit changes as a figure-target relationship is established to resemble the activity in response to the well-known stimulus.

Major Findings:

1. Controversy concerning the existence and nature of SM topography has stemmed from methodological problems associated with electrical stimulation of SM. Results from previous microstimulation studies of SM have led to the suggestion that this area lacks a motor topography. The new methodological approach, outlined above, has yielded a topographic map of motor organization. In this map, orofacial movements, including movement of the pinnae, lips, tongue, and jaw, and conjugate eye movements to the contralateral visual hemifield, were observed most rostrally. Adjacent to and overlapping with this orofacial and eye movement region was a region from which forelimb movements were evoked. Evoked forelimb movements usually included action at 2 or more joints. Caudal to and overlapping the sites evoking forelimb movements was a third region of the SM from which hindlimb and tail movements were evoked. The hindlimb and tail region of SM merged with the hindlimb region of MI without a distinguishable boundary. The overall rostrocaudal extent of the SM covered 12-14 mm.

The region of frontal cortex over which saccadic eye movements can be evoked with low stimulus currents had been thought to be limited to the FEF and, based on our recent results and those of others (Schlag and Schlag-Rey, *J. Neurophysiology*, 1987, 57:179), rostral SM. Using the new stimulation technique, however, we have

evoked eye movements from sites along a thin strip of cortex extending between these two fields. Movements evoked nearer the FEF were most like those evoked from the FEF, saccades of a fixed displacement and direction, regardless of initial ocular position. Closer to SM, evoked movements were most like one class described by Schlag and Schlag-Rey for the SM, eye movements depended upon the initial ocular position and always terminated near the same final position. Thus the FEF and rostral SM eye movement areas may be part of a larger cortical area concerned with eye movement control.

Preliminary simulation in and around the arcuate sulcus indicate that forelimb movements can be reliably evoked from PM. A detailed topographic analysis of the evoked movements must await the results from the histological examination in progress.

2. Quantitation of cell sizes along the medial wall of the cortex has allowed an objective evaluation of the cytoarchitectonic transition from area 4 to area 6. The rostrocaudal distribution of cell bodies greater than $600 \mu\text{m}^2$ and of cell bodies greater than $1200 \mu\text{m}^2$ show that these populations decrease from a peak to low densities over a 4 to 5 mm range. Neurons with areas of $300\text{-}500 \mu\text{m}^2$ do not have the same rostrocaudal change in density. The qualitatively-determined boundary between area 4 and area 6 corresponds to the rostrocaudal level at which the largest pyramidal cells appear in substantial densities. The location of this boundary determined relative to the tail representation (of MI, SM, or both) agrees well with that described previously.

3. Findings from the first single-unit study was inconclusive in one monkey. The task chosen, while appropriate for dissociation of direction of hand displacement from the direction of wrist rotation, could not test the experimental question. Electromyographic analysis of the flexion/extension task showed that the extensor carpi radialis muscle is active for upward movements in both postures. Therefore it is not possible to conclude that cells discharging before upward hand movements, independent of the direction of movement around the wrist, code for spatial trajectory of hand movement, as hypothesized for PM. This problem of interpretation points out the difficulty of behavioral neurophysiological work in the distal forelimb. On the basis of this experience, the principal investigator decided to abandon this approach, and the monkey was studied instead in an anatomical paradigm developed by Charles Gerfen, LCB/NIMH.

Significance to Biomedical Research and to the Program of the Institute:

The project represents an effort to explore the organization of the frontal cortex. SM is the primary cortical target of the basal ganglia. Examining the internal organization of PM, FEF, and SM, and their interactions with the other frontal areas is of importance in gaining an overall understanding of the frontal cortex and its role in health and disease.

Proposed Course of the Project:

The current project involves a systematic study of three motor areas, MI, SM, and PM and the frontal cortex areas involved in eye movements. Before the start of this project, the somatotopy of MI had been well established. Stimulation mapping has demonstrated SM topography and a heretofore unstudied eye movement area.

The first priority is to complete PM stimulation mapping. Further work on PM topography will depend upon the outcome of this mapping study. If the results from stimulation show that the PM forelimb representation resides between the FEF and the forelimb representation of MI, then the mapping work will be complete. If, however, the maps contradict current ideas concerning PM organization, the stimulation mapping can be combined with an anatomical tracer study. In such a study either the forelimb or the hindlimb representation of PM and MI are elucidated with electrical stimulation mapping and then tracer dyes are injected into the representations. This combination anatomical and physiological methods to study PM topography in macaque monkeys is possible with the new stimulation method described above.

Another possible line of study would be to explore the eye movement area found between the rostral SM and the FEF. As discussed above, some ocular movements evoked by stimulation in this area depend upon the initial eye position. It would be valuable to test the visual receptive fields of isolated single units with the eyes in different positions to see if some units have different receptive fields with different eye positions. However, eye movement studies are most easily studied with the aid of scleral search-coil oculometry, a technology not immediately available in this laboratory.

A new project is underway to record single-unit activity in the PM during movements in response to learned stimuli. As discussed above, PM may be involved in recall of motor programs. If this can be shown, a central role for PM in the generation of learned movements will have been elucidated. Further experiments would be aimed at examining the transition from the unlearned to the learned condition. If units in the PM are insensitive to the difference between novel stimuli and those associated with a specific motor response, then current interpretation of PM lesion experiments (by others) must be re-examined.

Publications:

Mitz, A. R. and Wise, S. P. (1987) The somatotopic organization of the supplementary motor area: Intracortical microstimulation mapping. J. Neuroscience, 7:1010-1021.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01097-01 LNP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Activity of Corticostriatal Neurons in Motor Cortex of Primates During Wrist Movement

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Melvyn P. Heyes Visiting Associate LNP, NIMH

Others: Steven P. Wise Research Biologist LNP, NIMH

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TOTAL MAN-YEARS:

0.4

PROFESSIONAL:

0.4

OTHER:

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- (a) Human subjects (b) Human tissues (c) Neither
- (a1) Minors
- (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although it is well known that the major input to the striatum originates in the cerebral cortex, virtually nothing is known about the type of information conveyed by the corticostriatal pathway. The part of the striatum that receives input from the primary motor cortex is the putamen. Putamen neurons discharge at or after the onset of EMG activity and reflect the direction of movement per se, whereas many neurons in the primary motor cortex discharge prior to the onset of EMG activity and reflect individual muscle activity independent of the direction of movement. To investigate corticostriatal (CS) activity during movement, we developed a strategy to identify CS neurons and quantitate their activity, their timing with respect to movement, and their relationship to the pattern of muscle activation in rhesus monkeys. Our observations indicate that motor information is conveyed via the CS pathway and that CS neurons more closely resemble primary motor cortex neurons than putamen neurons with respect to their timing and relationship to the patterns of muscle activity. It is possible, therefore, that motor disorders could result from excess in CS activity that causes abnormal contraction of muscles and limb position in space.

Objectives:

Anatomical studies have established that the major afferent input to the striatum originates in the cerebral cortex. However, virtually nothing is known about the type of information that may be conveyed by the corticostriatal (CS) pathway. The purpose of this project, then, was to develop a strategy of identify CS neurons in the cortex and evaluate the type of information they conveyed to their targets. Because movement is a very well defined and easily quantifiable output of the brain, the activity of CS neurons in the motor cortex was monitored during the performance of a simple motor task.

Electrophysiological studies have shown that movement-related modulations of neuronal activity in the putamen begin only briefly before movement begins, occurring at the same time or after the earliest muscle EMG activity causing the movement. In contrast, many neurons in the motor cortex, particularly those projecting through the pyramidal tract, begin their activity modulations substantially before the earliest changes in muscle EMG activity associated with movement. In addition, neuronal activity in the putamen is relatively unaffected by torque loads assisting or opposing movement, whereas the activity of neurons in the motor cortex are significantly modified by loads. In this respect the output of the motor cortex reflects the activity of individual muscles during the movement, whereas putamen neurons tend to reflect the pattern of movement. For example, when a flexion movement is performed against a load, agonist muscle activity is potentiated compared to when the same movement is performed against no load. In contrast, if the load applied assists the movement, the agonist muscle no longer may be active and the actual movement may be executed by regulation of antagonist muscle activity only. In this situation, motor cortex neuronal activity would be potentiated by opposing loads and attenuated by assisting loads, whereas neuronal activity in the putamen would be relatively insensitive to the loads. The activity of CS neurons under these circumstances was the subject of the present study.

Methods:

Two adult male rhesus monkeys were trained to make repeated flexion and extension movements of their right wrist between three target windows (A, B and C) each 8° wide in a repeated A,B,C,B,A sequence. The monkey viewed a line of 32 red light emitting diodes (LED) 16 above and 16 below a large green LED. When the wrist was positioned within the correct window, the green light was turned on. Movement to the next window was initiated after 750 ms, 1000 ms or 1250 ms (randomly selected) period following a reward (see below) by turning off the green LED and turning on a red LED whose direction and distance from the green LED informed the monkey of the direction and magnitude of the required movement. A correct movement was defined as a movement to the designated window within 750 ms of the trigger signal and maintenance of that position for 1500 ms; a movement in the wrong direction or an overshoot of the target window by more than 3 degrees triggered a return to the original window and a repeat of the trial. Correct movements were rewarded with fruit juice (0.1 ml). Once each monkey had mastered the paradigm, (>67% success) torque loads were applied.

The timing of movement with respect to neuronal activity was monitored. In addition the effects of torque loads that either assisted or opposed movement in a particular direction were tested. Studies of EMG activity during load application showed that assisting and opposing loads attenuated and accentuated, respectively, the EMG activity of agonist muscles.

The recording electrode was slowly advanced through the cortex while the putamen was being stimulated once every second. This strategy allowed detection of field potentials and unit responses from the CS neurons. When a putative CS neuron was detected, it was isolated and the discriminated signal used as trigger for the stimulation of the putamen. A neuron was accepted as a CS neuron when their response was absent following a collision with a spontaneously occurring cell discharge within its collision window and when the response to putamen stimulation at 333 Hz followed each of five stimuli with a constant antidromic latency.

Once a CS neuron had been unequivocally identified, its activity was studied during movement. In monkey #1, the highest priority was to determine the timing of movement-related discharges of CS neurons. In monkey #2, the priority was to determine the effects of assisting and opposing loads on unit activity of movement-related CS neurons.

Major Findings:

Three principal findings have emerged from the analysis of corticostriatal cell activity: they discharge in relation to movement, the changes in their discharge rates precede muscle EMG activity, and their activity during steady state holding and movement is significantly affected by loads both with and against the direction of movement.

1. Relationship to movement.

A total of 48 CS neurons were identified in the first monkey and 46 were identified in the second monkey. The antidromic latencies in the two monkeys were comparable 1.12 ± 0.40 (SD) ms and 1.36 ± 0.51 ms. Assuming a length of 30 mm for the CS axons, the conduction velocity of our sample averaged 16 m/s.

Forty-nine CS neurons were isolated long enough to record their activity during the wrist flexion/extension task. Neuronal activity changes were related to wrist movement in 25 of these neurons, 11 in the first monkey and 14 in the second monkey.

2. Timing of activity changes.

EMG activity and the timing of CS cell activity modulations were studied most extensively in the second monkey. EMG records were obtained during the recording sessions and again after the completion of the single-unit data collection. The increase in EMG activity of the earliest prime-mover muscles preceded movement onset by 93 ms. The time from the premovement change in firing rate (unit activity onset) to the onset of movement was 120 ± 32 ms in 11 CS cells from the first monkey (range 52 to 175 ms) and 110 ± 31 ms in 14 CS neurons from the second

monkey (range 50 to 150 ms). Of the 13 CS neurons in the second monkey that we were able to study in detail, 8 were clearly active before the earliest task-related changes in EMG activity.

3. Effects of assisting and opposing torque loads.

Analysis of the effects of torque loads on the activity of CS neurons during movement is in the early stages and will be completed near the end of this fiscal year. However, some results have emerged from our preliminary analysis. During steady-state holding and movement, tonic and phasic discharge rates respectively were increased by loads against the preferred direction of wrist movement in 15 of 25 CS neurons.

Significance to Biomedical Research and to the Program of the Institute:

Studies of the functional relationship between the cerebral cortex and the striatum are important to understanding how abnormalities in the balance of striatal neurotransmitters could cause clinical symptoms. Diseases such as torsion dystonia, Huntington's disease and glutaric aciduria type I may be viewed both as an inappropriate contraction of muscle groups producing an abnormal positioning of the limbs and trunk in space. The physiological properties of the CS and putamen neurons suggest that these symptoms may reflect an imbalance in CS and/or putamen neuronal activity. In Huntington's disease, it is of interest that psychiatric symptoms develop first and motor disturbances appear once neural degeneration is well under way. Thus, the physiological interaction of frontal cortex with the striatum is of fundamental importance in understanding a variety of brain abnormalities that adversely affect mental health.

Proposed Course of the Project:

Data collection has been completed on this project, and analysis will continue into the next year. Once neurophysiological and histological analyses are complete, we intend to prepare the results for publication. No further data collection is intended for this project.

Publications:

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01098-01 LNP

PERIOD COVERED

February 1, 1987 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Anatomical Analysis of Neuronal Circuits

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Chisato Asanuma Stanfield Staff Fellow

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TOTAL MAN-YEARS:

0.67

PROFESSIONAL:

0.67

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Previous studies of the dendritic structure of neurons have predominantly relied on the century-old Golgi methods and the technique of injecting biological markers into single neurons in vivo. For technical reasons, however, it has been difficult to effectively combine these methods with other neuroanatomical labeling techniques. The recently introduced method of intracellularly injecting a fluorescent dye, Lucifer yellow, in fixed tissue slices is capable of providing a complete picture of neuronal geometry and has the potential for use in conjunction with other neuro-anatomical techniques. This project involves intracellular injection of Lucifer yellow in combination with retrograde labeling techniques and immunohistochemistry, and its objective is to extend the analysis of neuronal connectivity and transmitter specificities to a finer level than previously possible. Initially two questions will be addressed: (1) is there a point-to-point reciprocity between thalamo-cortical axon collaterals that innervate the thalamic reticular nucleus and the neurons in that nucleus which project back to the thalamus; and (2) where on the dendrites of reticular nucleus neurons do thalamocortical axon collaterals terminate?

Objectives:

This project is designed to elucidate, at the single-cell level, the structural details of the dendritic arborizations of identified neurons and the exact locations of identified afferent terminations on those neurons. The specific objectives are: (a) to develop the necessary techniques; (b) to determine the degree of reciprocity between thalamocortical axon collaterals that innervate the thalamic reticular nucleus with the neurons in the reticular nucleus that project back to the thalamus; and (c) to describe the exact distribution of thalamic afferents on the dendrites of reticular nucleus neurons.

Methods:

This investigation involves the development of the methods for combining intracellular Lucifer yellow injections with immunohistochemistry and with the retrograde transport of fluorescent dyes, and applications of the method to a series of studies on thalamocortical circuitry in rats.

Initially, selected foci in the ventral lateral nucleus or the mediodorsal nucleus of the thalamus are injected simultaneously with the retrogradely transported fluorescent dye, fluoro-gold, and the anterogradely transported plant lectin, *Phaseolus vulgaris* leucoagglutinin (PHA-L). These thalamic nuclei have been selected since, together, they represent the most prominent thalamic inputs to the frontal cortex. Following an appropriate survival period, the rats are perfused by a buffered aldehyde fixative solution and the brains sectioned on a vibratome. Selected sections are secured on glass slides and examined in a fluorescent microscope. Neurons in the thalamic reticular nucleus retrogradely labeled with fluoro-gold are impaled under visual guidance with glass micropipettes containing a 2% Lucifer yellow solution. As the dye is iontophoresed into the cell, it quickly diffuses throughout the neuron's dendritic arbor. Following the injection procedure, the sections are removed from the slides and run through standard immunohistochemical procedures for visualization of the anterogradely transported PHA-L, using rhodamine isothiocyanate as the label on the secondary antibody.

Major Findings:

The efforts of this project thus far have been devoted to establishing the necessary facilities and testing, successfully, the feasibility of the study. A series of experiments have been conducted in rats in order ascertain that the use of the retrograde tracer fluoro-gold, immunohistochemical procedures for visualizing the plant lectin PHA-L, and the intracellular Lucifer yellow filling procedure are compatible with each other in the same tissue. Further, several technical parameters have been assessed, such as the ratio of paraformaldehyde to glutaraldehyde in the fixative mixture required to optimize the intracellular filling of neurons with Lucifer yellow while not compromising the subsequent immunohistochemical results, the perfusion parameters, the temperature at which the tissue should be kept at for obtaining optimal filling of neurons with Lucifer yellow, and the types of stabilization precautions which are needed on the microscope in order to be able to maintain the pipette within the neuron long enough to inject sufficient amounts of Lucifer yellow.

The preliminary experiments conducted thus far indicate that this study is feasible. Retrograde labelling of neurons with fluoro-gold, intracellular injections of neurons with Lucifer yellow, and anterograde labeling of axon terminals with PHA-L immunohistochemistry are all compatible with each other in the same tissue and the use of each of these techniques do not compromise the effectiveness of either of the other techniques.

One slight problem which we have encountered has been that the fluoro-gold and PHA-L have a tendency to precipitate each other out of solution if mixed together. As a result of this, we have had to modify our procedures to make the initial injection of the fluoro-gold and of the PHA-L separately through double barrelled pipettes instead of together through a single barrel pipette.

Although we have most of the necessary equipment set up and ready for these experiments, we are still awaiting the arrival of a fluorescent microscope. We expect to receive it before the end of this fiscal year.

Significance to Biomedical Research and to the Program of the Institute:

A knowledge of the structural details of each of the circuits interconnecting the thalamus and cortex is an important step in furthering our understanding of how these circuits process neural information and mediate higher brain functions. This knowledge includes the dendritic geometry of identified neurons, their axonal targets, the location, on the dendrites, of identified inputs, and the transmitters specific to the neurons studied.

The traditional Golgi methods and the technique of injecting horseradish peroxidase (HRP) into single neurons have, in the past, been the predominant means used for examining the dendritic architecture of neurons. However, it has been difficult to effectively use these methods in conjunction with other tracing or immunohistochemical techniques. The recently introduced fluorescent dye, Lucifer yellow, designed and first synthesized by Mr. Walter Stewart of the Laboratory of Analytical Chemistry, NIADK, diffuses quickly throughout the cell into which it is injected and allows the visualization of the cell's entire dendritic arbor. This method of visualizing the dendritic structure of neurons has the potential for use in conjunction with a variety of other neuro-anatomical techniques, and should bring our understanding of the structural details of neuronal circuits to a finer level than possible in the past.

While a comprehensive understanding of all structural details inherent in any given neuronal circuit, along with an understanding of their significance in the context of specific functions, remains a major challenge, especially for those neuronal circuits mediating higher functions of the type which are relevant to mental diseases, a systematic and detailed structural study of the thalamic circuits underlying the transmission of external signals from subcortical centers to the frontal cortex should provide some clues concerning some basic input modulating mechanisms such as the control of attention and arousal. Both of these neural functions are, of course, quite relevant to many mental illnesses, and several recent hypotheses put forth suggest that neural circuits in the thalamus may play a critical role in these functions.

Proposed Course of the Project:

The initial focus of this project will be on the interrelation of the thalamic reticular nucleus with other parts of the thalamus. Although long held to be the final link in the ascending 'non-specific' activating system mediating the desynchronization of the cortical EEG (*i.e.*, arousal), the results of recent studies indicate that the thalamic reticular nucleus may instead be part of a feedback circuit that modulates the transmission of signals through specific thalamic relay nuclei. This feedback circuit has been postulated to be involved in the modulation of the relative amplitude of externally generated stimuli (Ahlsen et al., Exp. Brain Res., 1985, 58:134) and in the control of selective attention (Skinner and Yingling, Prog. Clin. Neurophysiol., 1977, 1:30; Crick, Proc. Natl. Acad. Sci., 1984, 81:4586). However, some investigators continue to maintain that the thalamic reticular nucleus mediates general levels of arousal (Steriade et al., J. Neurosci., 1986, 6:68). Since neurons in the thalamic reticular nucleus receive inputs from collaterals of thalamocortical relay axons and since neurons in the thalamic reticular nucleus project back to the thalamus, a study combining the retrograde transport of fluorescent dyes and the antero-grade transport of the PHA-L from the thalamus with injection of Lucifer yellow into thalamic reticular nucleus neurons is ideally suited for evaluating whether the specificity that would be predicted of a circuit involved in the modulation of selective attention exists in this particular circuit.

Upon successful completion of the first set of experiments, the project will be extended to include some investigations of cortical neurons. In particular, we will study those cortical neurons that project back to the thalamus and other neurons that project to the opposite cerebral hemisphere. In each case, the reciprocal afferents which would be pre-labeled with PHA-L (the thalamocortical axon collaterals in layer 6 of the cortex, and the terminal arborizations of commissural axons, respectively) are known to arborize selectively in quite close proximity to the corresponding cortical efferent neurons, although the details of these relationships are, at present, unclear. A fine grained anatomical study of the type outlined above, directed at these cortical efferent circuits should provide a more comprehensive picture of the details of these relationships, and could further our understanding of the functional significance of the cortico-thalamic feedback pathway and our understanding of interhemispheric information transfer.

Publications:

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01099-01 LNP

PERIOD COVERED

October 1, 1986 to September 30, 1987

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurochemical Interactions Between Cortical and Striatal Dopaminergic Activity

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Melvyn P. Heyes	Visiting Associate	LNP, NIMH
Others:	Ikuro Namura	Visiting Fellow	LCS, NIMH
	Stephen Suomi	Chief	LCE, NICHD

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Animal Center, Poolesville, Maryland 20837

TOTAL MAN-YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

CHECK APPROPRIATE BOX(ES)

- (a) Human subjects (b) Human tissues (c) Neither
 (a1) Minors
 (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Disturbances in the balance of regional brain dopaminergic activity have been implicated in the symptomatology of schizophrenia. Recent studies suggest that dysfunction of the dorsolateral prefrontal cortex (DLPFC) may cause the impaired motivation, shallow affect, and deficiencies in the performance of problem solving tasks suffered by schizophrenics. Similar deficits are produced in primates by dopamine depletion in the DLPFC. Together these observations suggest a role for DLPFC dopaminergic neurons in cognitive functions subserved by this area. In one series of experiments in rats, dopamine depletion in the medial frontal cortex (MFC), a possible rodent homologue of the DLPFC of primates, resulted in increased dopaminergic activity in striatum. Our observations in rats showed that dopamine depletion in the MFC by local injection of MPP⁺ or 6-OHDA was associated with increased dopaminergic in nucleus accumbens, but not the caudatoputamen. This project will now be extended to primates to further test the hypothesis that corticostriatal abnormalities underlie psychotic disorders in a species more closely related and similar to humans.

Objectives:

Disturbances in the balance of regional brain dopaminergic activity have been postulated to be responsible for the clinical manifestations of schizophrenia. This hypothesis stems from the fact that drugs that are effective in treating the hallucinations and delusions of schizophrenia are dopamine (DA) receptor antagonists. The observation that the 'negative features' of schizophrenia are exacerbated by DA receptor antagonists, is consistent with this notion.

Schizophrenics have impaired motivation, shallow affect and deficiencies in the performance of problem solving tasks. While they perform such tasks, schizophrenics display a paucity in blood flow in the dorsolateral prefrontal cortex (DLPFC) (Weinberger et al: Arch Gen Psychiatr. 43: 114, 1986). Somewhat comparable behavioral deficits result in humans following lesions in the DLPFC and in primates following depletion of DA from the DLPFC (Bronzoski et al: Science 205: 929, 1979), thus suggesting a primary role for the DA system in cognitive functions subserved by DLPFC.

In rats, DA depletion in the medial frontal cortex (MFC) is associated with increases in DA concentration in the caudatoputamen (CP) and nucleus accumbens (Pycock et al: Nature 286: 74, 1980), as well as that of its metabolites DOPAC and HVA. If the dopaminergic system of the MFC of the rats is analogous to dopaminergic system in the DLPFC of primates, and if DA depletion in the DLPFC results in increased dopaminergic activity in subcortical dopaminergic systems in humans as it does in rats, then it is possible that the positive features of schizophrenia result from increased dopaminergic activity in the CP or nucleus accumbens (Bannon and Roth: Pharmacol. Rev. 35: 53, 1983; Weinberger et al: 1986).

To date the results of Pycock and co-workers have not been reproduced in rats or investigated in primates. Should a reciprocal relationship between frontal cortex and subcortical dopaminergic activity be demonstrated in primates, these animals may serve as a primate model for schizophrenia.

In this fiscal year, a preliminary study in rats was conducted in collaboration with Dr. Ikuro Namura of the Laboratory of Clinical Science. DA was depleted in the MFC by local injections of either 6-hydroxydopamine (6-OHDA) or N-methyl-4-phenylpyridine (MPP⁺).

Methods:

DA was depleted in the MFC of adult male Sprague-Dawley rats by bilateral injections of either MPP⁺ or 6-OHDA into the MFC. Saline injection served as controls. All of the 6-OHDA and half of the MPP⁺-treated rats received an intraperitoneal injection of desmethylimipramine (DMI) thirty minutes prior to the MFC injection in an attempt to protect noradrenergic neurons. The concentration of DA, DOPAC, homovanillic acid (HVA), norepinephrine (NE), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) was then measured by HPLC and electrochemical detection in MFC, the dorsal region of the cingulate cortex, striatum and nucleus accumbens two weeks later.

Major Findings:

Injections into the MFC were found to decrease DA levels both there and in the adjacent cingulate cortex.

In the caudatoputamen, DA, DOPAC and HVA concentrations were unaffected in any group. In MPP and DMI treated rats, DA concentration were increased by 22.8%. However, the large standard deviation rendered this increase statically insignificant. In MPP⁺+DMI treated rats the concentrations of DOPAC and HVA were also increased in CP by 11.2% and 27% respectively, but this also was not significant. No changes in the concentration of NE, 5-HT or 5-HIAA was observed in the CP.

In contrast, in the nucleus accumbens, DA concentrations were increased in the MPP⁺ group by 18.5% and in MPP⁺+DMI treated rats by 62.8%. DOPAC and HVA concentrations were also increased in these groups to similar extents. 5-HT concentrations in nucleus accumbens were increased in MPP⁺ treated rats but decreased in 6-OHDA treated rats. No changes in NE or 5-HIAA were observed in the nucleus accumbens.

Significance to Biomedical Research and to the Program of the Institute:

This project tests one hypothesis about the cause of schizophrenia and include an attempt to develop an animal model of the disease. In the nucleus accumbens of both groups of MPP⁺-treated rats, significant increases in the concentrations of DA, DOPAC, and HVA were found, indicating increases in dopaminergic activity. This is consistent with the hypothesis and encourages us to move up to a primate model.

Proposed Course of the Project:

Dopamine will be depleted in the DLPFC and medial prefrontal cortex (MPFC) of monkeys by local injections of either 6-OHDA or MPP⁺. These regions have been chosen because of their high DA content, their inputs from the VTA and the likelihood that one of these regions is the homologue of the rodent MFC. Six rhesus monkeys, that have been raised under closely monitored social conditions, will be studied in the initial two groups.

Key behavioral observations are planned in collaboration with Dr. Stephen Suomi, LCE, NICHD. These include a study of the effect of regional frontal dopamine depletions on social hierarchies and their effect on affiliative behavior.

Publications:

None.

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