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DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1991 - September 30, 1992

VOLUME II PART 2

INDIVIDUAL PROJECT REPORTS

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DIVISION OF INTRAMURAL RESEARCH PROGRAMS
NATIONAL INSTITUTE OF MENTAL HEALTH

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00478-36 LN

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*
Neural mechanisms of stimulus memory and habit formation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7.3	3.0	4.3

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☐ (a) Human ☐ (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 subcortical circuits has reciprocal connections with one pair or the other of the cortical cell 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 stimulus memory, both of which can be distinguished from habit formation. The latter form of learning involves stimulus-response association and depends largely on interactions between the cerebral cortex and the basal ganglia.

PROJECT DESCRIPTION:

Behavioral analyses of the effects of selective cerebral ablations and disconnections, together with anatomical analyses of functional neural pathways, are used to determine how object and spatial perceptions in the different sensory modalities are formed into memories, how these different memories are associated with each other, how they evoke emotions and motor acts, and how perceptions lead not only to these cognitive events but also to habit formation.

(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).

To date, experiments assessing the roles of the amygdala and hippocampus in recognition memory have been complicated by the fact that removal of these limbic structures also involves removal of the ventrally-adjacent rhinal cortex (Rh), which lines the banks of the rhinal sulcus. The Rh is comprised of the entorhinal cortex (ERh) and the perirhinal cortex (PRh), two areas that are intimately related to both the amygdala and hippocampus. Recently, we found that the Rh itself makes a critical contribution to recognition memory, either directly, by virtue of its connections with the neocortex and diencephalon, or indirectly, by relaying sensory information from modality-specific neocortical areas to other medial temporal lobe structures known to be important for recognition memory, the amygdala and hippocampus. Even more recently, in order to determine the relative contributions of ERh and PRh to recognition memory, we have examined the behavioral effects of lesions restricted to these cortical fields. The preliminary results indicate that whereas PRh makes a substantial contribution to recognition memory, ERh makes a relatively minor contribution. Further, because neither the PRh lesion nor the Rh (PRh plus ERh) lesion alone yields as severe a deficit as that observed following the combined amygdala, hippocampal plus Rh lesions, other relays must exist for transmitting information to medial temporal or medial diencephalic structures.

Recently, we have begun to examine the effects on recognition memory of hippocampal (H) removals combined with rhinal cortical ablations. An earlier study from this laboratory had indicated that monkeys with H + Rh lesions were no more impaired than

animals with H lesions, and not so severely impaired as monkeys with Rh lesions alone, but the Rh lesion employed in that study differed from the present Rh lesion in that it spared perirhinal cortex on the rostral surface of the temporal pole. The preliminary data indicate that monkeys with the new H + Rh lesions, which include this rostral perirhinal cortex, are indeed as impaired as monkeys with Rh lesions alone.

(2) Recency memory

Earlier studies had indicated that removals of the amygdala and subjacent cortex produce a severe deficit on a version of nonmatching-to-sample with two repeatedly used objects, and the impairment was attributed to the amygdala damage. In contrast to the version of delayed nonmatching with trial-unique objects, this version of delayed nonmatching, which employs the same two objects across trials, is difficult even for normal animals to learn, presumably due in part to the interference engendered by reinforcement of contradictory responses to each member of the pair. Further, whereas the version of delayed nonmatching with trial-unique pairs measures recognition memory (i.e. "which object was presented previously?"), the version with a single pair measures recency memory (i.e. "which object was presented most recently?").

In order to determine the critical neural substrate for recency memory, monkeys were trained on versions of delayed nonmatching with multiple object pairs and with a single pair. These monkeys then received selective ablations of either the anterior rhinal cortex (tissue which underlies the amygdala and is included in aspiration lesions of the amygdala) or the amygdala itself (by ibotenic-acid injections), or were retained as unoperated controls. The results indicate that the deficit on single-pair delayed nonmatching reported earlier to follow amygdala removals is due, at least in part, to anterior rhinal cortical damage. Further, because two of the three subjects with amygdala removals appeared to have complete lesions, we can conclude that the amygdala is not necessary for recency memory. In these same subjects, monkeys with anterior rhinal cortical lesions performed like the controls on a test of food preference whereas the amygdalectomized animals did not. This finding confirms the results of earlier investigators and is consistent with a role for the amygdala in mediating either crossmodal associations or affective responses to environmental stimuli.

(3) Associative memory

Monkeys with extensive medial temporal lesions that include the amygdala and hippocampus are unable to associate visual stimuli with other visual stimuli, as assessed by their performance on a visual-visual paired association task. In order to assess the

contribution of the Rh region to such recall memory, we tested monkeys with Rh ablations on the same task, and found them to be are impaired as severely as those with more extensive lesions. As in the case of recognition memory, additional studies are needed to elucidate how the rhinal cortex participates in associative memory, i.e. does it serve as a relay to the medial diencephalon directly or indirectly through the amygdala and hippocampus.

(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 controls 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 related examples of preserved learning ability following limbic-system lesions.

We have recently found that lesions monkeys with rhinal cortical lesions also perform well on this task, thus showing the same pattern of impaired recognition and spared habit formation as monkeys with lesions that include the amygdala and hippocampus. These data provide further evidence either that the rhinal cortex relays information to the medial temporal lobe limbic structures before a stimulus trace mediating recognition can be established, or, alternatively, that the rhinal cortex alone is sufficient. Further experiments are underway to decide between these two possibilities.

Recently, we have used the delayed nonmatching-to-sample (DNMS) paradigm as a measure of the monkey's ability to acquire habits. Although combined amygdalo-hippocampal (AH) removals in macaques severely impair their performance on DNMS when delays between sample and choice exceed about 10 seconds, they can master the task with shorter delays. To master the task in the absence of the limbic system, the animal must be able to learn a rule, which requires, in turn, (i) suppression of specific stimulus-response habits, (ii) abstraction of sameness and difference from specific stimulus quality with the aid of immediate memory, and (iii) formation of a stimulus/ difference-response habit. Recently, we have found that if inferior prefrontal cortex (IF) lesions (which themselves produce a moderate DNMS impairment) are added to AH lesions, monkeys lose the ability to perform DNMS even when the delays are shorter than 10 seconds. This finding suggests that IF serves one or more of the processes described above needed for rule learning.

To tease apart the contributions of the IF to those processes, we

evaluated the behavioral effects of the combined IF and AH removals on two additional tasks. The first task, delayed matching-to-sample (DMS), is almost identical to DNMS except that it does not require the suppression of specific stimulus-response habits (i, above). Preliminary results indicate that monkeys with the combined (but not separate) IF and AH lesions fail to learn DMS, just as they fail to relearn DNMS. Thus, the critical contribution of the IF to performance on DNMS cannot be linked solely to the process of suppression of specific stimulus-response habits.

In the second task, we removed the requirement for immediate memory but still required the same/different discrimination (see ii, above); the monkeys were given a choice between a like and an unlike pair of trial-unique objects that were presented simultaneously, with the like pair designated correct. Although impaired on the task, each animal with the combined IF and AH lesion was able to relearn it. Taken together, the results of these experiments suggest that the IF and AH each make an important contribution to same/different discrimination, but their combined contributions are essential only when immediate visual memory is also required.

(5) Cognitive and motor impairments associated with SIV infection

Over 50 percent of humans infected with the human immunodeficiency virus (HIV) exhibit cognitive, motor, or affective changes. Because little is known about the nature of central nervous system involvement in HIV-infected humans, we have examined rhesus monkeys, which, when infected with a simian immunodeficiency virus (SIV), develop an immunodeficiency disease similar to that produced by HIV.

Rhesus monkeys' performance on cognitive and motor tasks was evaluated both before and after either SIV (N=10) or sham inoculation (N=5). The tasks employed were delayed matching-to-sample with trial-unique stimuli (recognition memory), delayed matching-to-sample with two repeatedly used stimuli (recency memory), visual discrimination learning with 24-hour intertrial intervals (habit formation), and a motor skill task.

Eight of the ten virus-inoculated monkeys were productively infected based on determinations of virus rescue and serum antigen levels. Two to five months after inoculation, but before any evidence of opportunistic infection, four of the eight infected monkeys developed significant neuropsychological impairment on one or more of the behavioral tasks. One animal was impaired on both the recognition and recency memory tasks, as well as in discrimination learning and retention. Another animal was impaired on both discrimination learning and retention and on

the motor skill task. Each of the two other behaviorally affected monkeys exhibited impairment on one task only, one in discrimination learning and retention, and the other on the motor skill task. At this same time, the other four infected monkeys obtained scores that were in the range of those achieved by the control animals. By the end of testing, about 10 months after inoculation, all but one of the SIV-infected monkeys showed declines in scores on the motor skill task that were slight but nevertheless significant. No correlation could be discerned between any of these impairments and either the locus or extent of the neuropathology, which involved alterations ranging from slight perivascular lymphocytic inflammation and choroid plexitis to typical SIV-induced meningoencephalitis, with macrophages and multinucleated (syncytial) giant cells. There was a significant correlation, however, between the time of onset of the motor skill impairment and the time of peak quinolinic acid levels in the cerebrospianl fluid of the infected monkeys. No such relationship was found between quinolinic acid levels and cognitive impairment. This may provide a key to the mechanisms underlying at least some aspects of the neuropsychological dysfunction observed in humans and monkeys infected with HIV and SIV, respectively.

These data demonstrate that SIV-infected rhesus monkeys, like HIV-infected humans, develop selective cognitive and motor deficits. This dysfunction closely resembles that seen in HIV-infected humans in the nature of the neuropsychological impairment, the proportion of affected individuals, the variable pattern of impairment, and the apparent lack of correlation with neuropathological findings. The neuropsychological status of SIV-infected rhesus monkeys may prove to be valuable bioassay allowing not only for the identification of the agents causing the neural dysfunction, but also for evaluation of putative anti-viral agents.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

These findings provide new insight into the neural substrates of perception, memory, concept formation, and habit formation, thereby helping to elucidate the organization of memory and other cognitive processes. A better understanding of these processes will inevitably lead to a better understanding of normal thought processes and of their breakdown with brain injury or disease.

PROPOSED COURSE OF RESEARCH:

We shall continue to investigate: i) whether the limbic system is critical for recognition and recall in all perceptual modalities; ii) whether we can differentiate between amygdalar, hippocampal, and rhinal cortical contributions to associative memory; iii) whether any functional contributions of these temporal lobe

structures are carried further through the thalamic, prefrontal, and neurochemical segments of the two limbic circuits; and iv) the neural basis of habit formation, with particular attention initially to the neostriatal and prefrontal targets of the occipitotemporal visual system. In addition, we plan to continue the study of SIV-inoculated rhesus monkeys to determine what agents are responsible for the cognitive and motor deficits associated with infection.

PUBLICATIONS:

Alkon, D.L., Amaral, D.G., Bear, M.F., Black, J., Carew, T.J., Cohen, N.J., Disterhoft, J.F., Eichenbaum, H., Golski, S., Gorman, L.K., Lynch, G., McNaughton, B.L., Mishkin, M., Moyer Jr., J.R., Olds, J., Olton, D.S., Otto, T., Squire, L.R., Staubli, U., Thompson, L.T., and Wible, C. Learning and memory. Brain Res. 16: 193-220, 1991.

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Kowalska, D.M., Bachevalier, J., and Mishkin, M. The role of the inferior prefrontal convexity in performance of delayed nonmatching-to-sample. Neuropsychologia 29: 583-600, 1991.

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Murray, E.A. Medial temporal lobe structures contributing to recognition memory: the amygdaloid complex versus the rhinal cortex. In J.P. Aggleton (Ed.): The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. Wiley and Sons, New York, pp. 453-470, 1992.

Murray, E.A., Rausch, D.M., Lendvay, J. and Eiden, L.E. Cognitive and motor impairments associated with SIV infection in rhesus monkeys. Science 255: 1246-1249, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02032-16 LN

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*
Neural coding of visual stimuli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

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	J.W. McClurkin	Staff Fellow	LSR NEI

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

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

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

We recorded the activity of single neurons in the lateral geniculate nucleus, primary visual cortex (the first cortical stage of visual processing), and inferior temporal cortex (the last cortical stage), to study the mechanisms underlying visual perception. When the responses were analyzed for the information they contained, we found that the response patterns could be represented as the sum of several (3-6) simultaneous, independent patterns of activity. These activity patterns were analyzed as a temporal code, and this code was found to contain more information than that conveyed by the response strength, the usual measure of neuronal response. Conceptualizing the neuron as having several independent activity patterns allowed us to use the responses elicited by one set of visual patterns to predict those elicited by other patterns. We also found that neurons in inferior temporal cortex recorded from while monkeys perform a delayed matching-to-sample task carry stimulus-specific information differentiating the pattern stimuli, and reflecting whether a given stimulus is the sample, the match, or the nonmatch. The change in the neuronal response across conditions for a few stimuli could be used to predict the change in neuronal response to other stimuli across those same conditions. Inferior temporal neurons also carry information about the stimulus being searched for (i.e. the sample) when the stimulus is either the match or nonmatch. Using a different model we were able to show how the responses to the stimulus being viewed were modified by the responses to the preceding stimulus. Each member of a neuronal pair recorded from simultaneously with a single electrode in a given cortical visual area is independent of the other, i.e., their information adds. This suggests that local processing is carried out by adding independent analyses of the stimulus being viewed.

PROJECT DESCRIPTION:

Objectives:

Perception and recognition of complex visual patterns arise from the cooperative properties of the single neurons within the visual system. To study these neurons, we conceptualize them as communication channels that transmit information about visual patterns and their significance. We have investigated the structure of neuronal codes within the visual system using techniques from signal processing, statistics, systems analysis, and information theory. Using this approach we discovered that single neurons throughout the visual system carry multiple, simultaneous, independent messages about visual stimuli multiplexed in a temporally modulated response code.

Major findings:

During this past year we concentrated on three lines of work related to neuronal codes. First, we continued exploring the relation between neurons that are recorded from simultaneously with a single microelectrode. Two conflicting hypotheses about the functions of local neurons are that they carry independent information, thereby increasing the richness of the messages, or that they carry redundant information, thereby increasing the reliability of the messages. We measured the amount of information carried about a stimulus set in simultaneously recorded pairs of neurons. The amount of stimulus-related information will be additive if the neurons are acting as independent channels, but it will increase by a multiplicative factor equal to the square-root of the number of neurons, e.g., a factor of 1.41 for two neurons, if the information is completely redundant and the noise is uncorrelated. We found that the information carried by two neurons is additive, i.e., the information in each is independent of that carried by the other. In addition we found that the noise in each neuron's response that is mixed with the signal also is independent. Our result indicates that the responses of one neuron can not be used to predict the responses of the adjacent one. This is surprising since it had been suggested previously that adjacent neurons frequently respond to most of the same stimuli, implying that the neurons must be directly connected or receiving many of the same input fibers. If this assumption is still correct, then the neurons must be acting on different information coming from the same inputs. Our results suggest that population codes that rely on simple averaging can not be the basis of function within local sets of neurons, because averaging would destroy the information carried by independent neurons.

Second, we have analyzed neuronal responses for evidence of oscillatory activity. Several groups of investigators have

recently suggested that the oscillations they found in the 30-70 Hz frequency range elicited by moving stimuli from neurons recorded from visual cortex in anesthetized cats might be related to linking features from one stimulus that fall on the receptive fields of different neurons. To test this proposal we recorded both neuronal action potentials and local field potentials from inferior temporal cortex in monkeys performing a pattern recognition task involving stationary two-dimensional black and white stimuli.

We found no relationship between the local field potentials and action potentials. In addition, we examined the information in the responses in the frequency bands of dc-22 Hz, 23-44 Hz, and 45-66 Hz, and found that the information about the stimulus in the lowest band, i.e. dc-22 Hz, 4 times greater than that in either of the other two bands. In addition, the information in the two higher bands was completely redundant with the information in the dc-22 Hz band. This absence of signs of coherent oscillatory activity supports our finding above that local neurons are acting independently.

Third, because lesions of inferior temporal cortex seriously impair pattern recognition in monkeys, we studied the information carried by neuronal codes related to pattern recognition. We presented a set of 32 black and white stimuli in a simple sequential pattern matching task, delayed match-to-sample. In this task, a stimulus is presented as a sample, and a short time later, 650 ms here, a second stimulus is presented for comparison. The monkey must make one behavioural response if the test stimulus matches the sample, and another if it doesn't. Thus, the same stimulus can take on three different meanings: sample, match, or nonmatch. We found that the responses were significantly modulated by both the pattern and its meaning. That is, the neurons carried significant amounts of information about the pattern (0.42 bits), the meaning of the stimulus (0.05 bits), and, in the nonmatch condition, information about the preceding sample stimulus (0.11 bits). These results suggest that inferior temporal cortex has signals related to at least two of the four steps, i.e., the comparison and decision steps, required for pattern recognition to occur. Applying network computation techniques to our data, we have constructed two models that can compare messages and help predict the responses of these neurons. One model is related to remembered stimuli, and one to stimulus meaning. In the first, a nonlinear model, the memory of a stimulus is kept as a response template that is compared (multiplied) by the current stimulus to predict the observed response. The predictions are quite good. In the second, a linear model, the context is also encoded within the responses on the basis of our observation that when the actual response messages were examined graphically, the messages about the three conditions, sample, match, and nonmatch, were found to

have shifted on the graph. We found that linear transformations (modeled as multiple regression) allowed very good predictions of the responses in one condition based on the responses in the other conditions.

We have now taken this analysis one step further. In collaborative work with a group at the Nordita Institute of Theoretical Physics we have continued to explore how well neuronal codes can be decoded. Using data from the delayed match-to-sample experiments described above, we trained computational networks or artificial neural networks to identify the meaning of the stimulus (i.e. sample, match, or nonmatch). Although our information theoretical analysis had shown that it should be possible to decode this information, information theory gives no clue as to the method needed, e.g. whether a simple model with relatively few degrees of freedom would be adequate. In fact, we found that an artificial neural network, which can be thought of as a simple piecewise mapping function, will perform this task, showing that the decoding rules can be embodied in a low-order model. We found in addition that this approach can form the basis for a simpler and more robust method to estimate the amount of information in a neural signal.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Disorders of attention, perception, and memory accompany most, if not all, psychiatric and many neurological disorders. This project studies how information is encoded and transmitted in the responses of single neurons with the ultimate goal of designing strategies both for more effective palliative treatment of cognitive deficits and for restitution of cognitive function.

PROPOSED COURSE OF RESEARCH:

We will continue the three experimental projects described above. With the use of new computational equipment, the data from those experiments will form the basis for simulations of the function of small and large groups of neurons to address questions about how information is combined to form stable percepts.

PUBLICATIONS:

Eskandar, E.N., Optican, L.M., and Richmond, B.J. The role of inferior temporal neurons in visual memory: II. Multiplying temporal waveforms related to vision and memory. J. Neurophysiol., in press.

Eskandar, E.N., Richmond, B.J., Hertz, J.A., Optican, L.M., and Kjaer, T. Decoding neural signals in visual pattern recognition. In J.E. Moody, S.J. Hanson and R.P. Lippmann (Eds.): Advances in Neural Information Processing Systems Morgan Kaufmann. San

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02035-12 LN

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*
Anatomy of the primate visual system

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

P.I.:	Ungerleider	Research Psychologist	LN, NIMH
Others:	M. Mishkin	Chief	LN, NIMH
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	M.J. Webster	Visiting Associate	LN, NIMH
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NIMH, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.3	3.0	3.3

CHECK APPROPRIATE BOX(ES)
☒ (a) Human ☐ (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 examined the functional areas that comprise this cortex in the macaque and explored their interconnections by the use of neuroanatomical tracing techniques in combination with physiological recording of neural activity. Our results indicate that a multiplicity of separate visual areas lie beyond the striate cortex (V1) in the stream of information processing. These areas are organized into two divergent cortical pathways, each having V1 as the source of its initial input. One, an occipitotemporal pathway, enables the recognition of objects, while the other, an occipitoparietal pathway, mediates the appreciation of spatial relationships among objects as well as the visual guidance of movement. The areas along the occipitotemporal pathway (V1, V2, V3, V4, and TEO and TE of the inferior temporal cortex) appear to be organized as a hierarchy, in which each area processes both color and form. By contrast, the areas along the occipitoparietal pathway (V1, MT, and MT's projection zones in parietal cortex) process the direction of stimulus motion. Visual information about object identity and spatial location, initially processed in separate cortical pathways, appears to be integrated in cortex located within the superior temporal sulcus. The subcortical connections of the two pathways, however, remain highly segregated. Whereas parietal but not temporal cortex projects to the pons and superior colliculus, temporal but not parietal cortex is reciprocally connected with the amygdala, indicating the importance of spatial information for the visual guidance of movement and of object identity information for object-reward associations. Data from cerebral blood flow studies indicate the existence in humans, as in monkeys, of two distinct visual processing pathways, although there may be cross-species differences in their precise anatomical locations.

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 their interconnections by the use of a variety of neuroanatomical tracing techniques in combination with electrophysiological recording of neural activity. 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 movement.

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. The major output of V4 is to a widespread region within the inferior temporal cortex, including area TEO posteriorly and area TE anteriorly. Mainly central field representations in V4 project to TEO, whereas both central and peripheral field representations in V4 project to TE. Because little is known about the properties of neurons within TEO, we mapped the area electrophysiologically and found that it contains a crude representation of the entire contralateral visual field. An especially high percentage of receptive fields recorded in TEO include the fovea, which is consistent with the major input this area receives from the central field representation of V4 and with the severe deficits in pattern perception that are seen following TEO lesions. In our recent studies of area TEO, we have found that this area relays to area TE visual information it receives from prestriate areas V2-V4. Because V4 also relays to TE input it receives from V2, we investigated the distributions of V4-projecting and TEO-projecting neurons within V2. The results showed that although TEO-projecting neurons are far sparser, they are intermingled with V4-projecting neurons; both of these classes of V2 neurons are located in cytochrome-oxidase rich thin stripes and interstripe regions, i.e., those regions with high proportions of color selective and orientation/length selective cells, respectively.

Neurophysiological studies in our lab have shown that V4 neurons process higher-order aspects of color and form. Similarly, our anatomical studies indicate that V4 plays an important role in relaying this information to object recognition mechanisms of area TE. To test this, we have studied the effects of V4 lesions

affecting a single visual quadrant, in monkeys trained to fixate.

Previously in this project, we had found that color and form perception are both impaired in the quadrant affected by the lesion, whereas motion perception is not, consistent with our anatomical results. We also found that the lesion caused a severe impairment in the ability to match colors or patterns across different locations on the retina, suggesting that V4 contributes to the mechanism for object equivalence across retinal translation, which is an essential property of normal object recognition. Although V4 lesions cause severe impairments in recognition, there remains some residual function. Correspondingly, we have now found that many TE neurons can still be activated following V4 lesions, and, thus, there must be an alternate anatomical route into the temporal cortex. Our recent anatomical finding of an indirect pathway from V2 to TE via area TEO (see above) would explain not only the partial sparing of color and form vision after V4 lesions but also the visual activation of TE neurons after such lesions. We have now begun behavioral studies to test the relative contributions of V4 and TEO to form and color perception.

2. An occipitoparietal pathway for spatial vision. We had previously found that the occipitoparietal pathway begins with projections from V1, V2, and V3 to area MT, which projects in turn to area VIP, located in the ventral intraparietal sulcus, and areas MST and FST, located on the medial bank and floor, respectively, of the superior temporal sulcus (STS). 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. We have found, additionally, that MST and FST send major projections to widespread regions of the posterior parietal cortex as well as to areas in the anterior STS. The latter region contains many cells with complex directional properties, such as sensitivity to rotation and optical flow. These results suggest that the cortical system for motion analysis, which begins with the projection of V1 to MT, splits into at least two components. One component includes regions of the posterior parietal cortex, whereas the other extends into the temporal lobe to include areas in the anterior STS. Thus, the neural mechanisms underlying visuospatial function may be far more extensive than previously thought. Recently, we have shown that the motion-analysis system might influence the control of eye and head movements through two parallel subcortical channels to the cerebellum, one via the pons and another via the nucleus of the optic tract, the lateral terminal nucleus, and the inferior olivary nucleus.

We have begun to assess the functional development of the

motion-analysis system using the 2-deoxyglucose method. Our preliminary results indicate that although this system is not fully mature at birth, it appears to mature faster than the occipitotemporal system for object recognition.

3. Interactions of the occipitotemporal and occipitoparietal pathways. To determine how the object and spatial information carried separately by the occipitotemporal and occipitoparietal pathways are integrated to yield a unified percept, we have been investigating possible anatomical sites of interaction. We have found that cells projecting to temporal and parietal cortex are located mainly in different extrastriate visual areas. However, two areas contain cells projecting to both, namely, V4 and the posterior bank and floor of the STS outside MT. In both V4 and STS, labeled cells projecting to the two destinations were found to be intermingled, though the projection to parietal cortex was heavier from the peripheral than from the central field representation of V4, whereas for the projection to temporal cortex the reverse was true. The results of injecting the temporal and parietal cortex with different anterograde tracers demonstrated that V4 provides feedforward information to both temporal and parietal cortex, whereas zones within the STS are sites for convergence of information from these regions. We have recently investigated the subcortical connections of the inferior temporal and posterior parietal cortex and have found that, like the cortical connections of the two visual pathways, the subcortical connections are remarkably segregated. Whereas parietal but not temporal cortex projects to the pons and superior colliculus, temporal but not parietal cortex is reciprocally connected with the amygdala. These results indicate, on the one hand, the importance of visuospatial but not object identity information for the visual guidance of movement, and, on the other, the importance of object identity but not visuospatial information for object-reward associations.

4. Chemoarchitectonic analysis of visual cortical pathways. Recent work has shown that a monoclonal antibody directed against a non-phosphorylated neurofilament protein, SMI32, labels pyramidal cells in monkey visual cortex in areal specific patterns. In V1, SMI32-immunoreactive (ir) cells are the ones projecting to MT, namely, those in layer 4B and the Meynert cells in layer 6. In V2, V3, V4, and MT, SMI32-ir cells are mainly in layer 2 and 3. Area MT is unique in having a band of SMI32-ir cells in layer 6. Because SMI32 antibody also labels alpha ganglion cells in the retina and the magnocellular cells of the lateral geniculate nucleus, SMI32 antibody may be a selective marker for components of the magnocellular pathway through the cortex. To test this possibility, we injected distinct fluorescent tracers into areas MT and V4 and analyzed the proportion of retrogradely labeled cells that were double-labeled with the SMI32 antibody. Our results indicated that, of the

cells projecting to MT, about 90% of those in V1 and 75% of those in V2 were SMI32-ir. By contrast, of the cells projecting to V4, less than 50% of those in V2 were SMI32-ir. These preliminary results are thus consistent with the predominantly magnocellular input to area MT and the relatively equal contributions of magnocellular and parvocellular inputs to area V4. Moreover, because SMI32-ir neurons are selectively lost in Alzheimer's disease, a selective vulnerability of cells receiving magnocellular inputs may explain the impairment in visuospatial function that is characteristic even in the early stages of the disease. This work was done in collaboration with members of the Fishberg Center for Neurobiology at the Mount Sinai Medical School.

5. Mapping visual processing pathways in humans. We have undertaken a collaborative study with members of the Laboratory of Neurosciences (NIA) to investigate whether there are separate visual pathways in human cortex for processing object identity and spatial location. In this study, regional cerebral blood flow was measured with positron emission tomography (PET) as normal subjects performed both an object identity and spatial location task. Areas activated more during the object than during the spatial task were located in occipitotemporal cortex, whereas areas activated in the spatial but not the object task were located in superior parietal cortex. These results demonstrate the existence in humans, as in monkeys, of two distinct visual processing pathways, although there appear to be cross-species differences in their precise anatomical locations. A correlational analysis between normalized regional blood flow values indicated that the processing of object identity and spatial location both depend primarily on functional interactions between posterior cortical areas in the right hemisphere.

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 tracing the complex system of projections stepwise from the primary visual cortex to the highest-order visual areas located within the temporal and parietal lobes, areas critical for object vision and spatial vision, respectively.

PROPOSED COURSE OF RESEARCH:

Our recent studies suggest that both the temporal and parietal lobes consist of multiple visual areas, and we will continue to investigate their organization. We will pursue the possibility of differentiating functional processing streams in continuing chemoarchitectonic studies. We also plan to investigate the

links of both visual processing pathways to affective and memory systems by examining the projections of the multiple visual association areas to limbic structures and the prefrontal cortex. Finally, in continuing PET studies, we will attempt to identify the multiple visual areas in the human cortex that have been differentiated in the monkey.

PUBLICATIONS:

Baizer, J.S., Desimone, R., and Ungerleider, L.G. Comparison of subcortical connections of inferior temporal and posterior parietal cortex in monkeys. Visual Neurosci., in press.

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Ungerleider, L.G. and Murray, E.A. Neural pathways for visual perception and memory in primates. In L.R. Squire, J.H. Byrne, L. Nadel, H.L. Roediger, D.L. Schacter, and R.F. Thompson (Eds.): Encyclopedia of Learning and Memory. Macmillan Publishing Co., New York, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 MH 02036-12

PERIOD COVERED
October 1, 1991 to September 30, 199

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*
Neural mechanisms for attention and memory in the extrastriate cortex

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

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

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

Disorders of perception, attention, and memory frequently accompany the major mental diseases. To understand the neural mechanisms of these mental processes, we are recording the activity of neurons in the extrastriate cortex and subcortical structures of monkeys engaged in tasks requiring visual discrimination, attention, and memory. We have developed neural network models that predict the responses of cortical neurons to complex patterns. We found that information processing in the cortex is modulated by selective attention, and that the source of the modulation is a network of critical structures, including the lateral pulvinar and the superior colliculus. Attention thus determines which visual information reaches neural mechanisms for memory storage. We have found evidence for neural mechanisms in the inferior temporal cortex that underlie both working memory and long-term memory. Inferior temporal neurons appear to function as adaptive mnemonic filters that compare current stimuli to stored memory traces. Further, the responses of neural populations in temporal cortex change as a result of experience, providing a possible basis for long-term memory. During recall of stored memories, specific neurons in inferior temporal cortex and area V4 are reactivated, thereby segregating, or "labeling", the neurons providing the information needed for a given task. In contrast to the mnemonic role of inferior temporal cells, cells in the caudal neostriatum show response changes related to the build-up of stimulus-response associations.

PROJECT DESCRIPTION:

This is a long-term project to understand the neuronal basis of perception and memory in extrastriate cortex as well as 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.

1. Neural network analysis of the primary visual cortex. Computer-simulated neural networks are a new tool for analyzing the role that nonlinear neurons play in large-scale networks such as exist in the visual cortex. As a first step, we have tested whether a neural learning algorithm can be used to train neural networks to simulate the responses of recorded cells to visual patterns. The cells were complex cells, and the patterns included not only a Fourier basis set but also arbitrary "real-world" patterns such as shaded 3-D surfaces and textures. These tests have been successful, in that the simulated networks predicted the responses of actual neurons to stimuli outside the training set, with a median correlation of 0.85 between the predicted and actual response. In addition, by inverting the network, we obtained an image of the "optimal" stimuli for the cells, images which were not included in either the training or test set. To our knowledge, this is the first successful use of a network to predict the recorded responses of nonlinear cells to realistic complex patterns. A paper reporting these results is now in press. We plan to apply this new technology to the problems of object recognition in extrastriate cortex.

2. A role for the superior colliculus in attentional control. Earlier in this project, we established that ignored stimuli are filtered out of the receptive fields of extrastriate neurons, explaining why we have little awareness of ignored stimuli. Our current task is to understand how this attentional control comes about. As a first step, we have tested the behavioral effects of reversible deactivation of structures that might be the sources of the neural signals that control cortical processing. The results show that both the lateral pulvinar and superior colliculus (SC) play a critical role in the ability to focus attention on a single stimulus and ignore distractors. However, previous neurophysiological studies have reported that the responses of SC cells are related to eye movements and not attention. To resolve this puzzle, we recorded or electrically stimulated cells in the superficial layers of the SC in monkeys performing attentional tasks. We found that SC cells give

enhanced responses to a stimulus if its location had recently been stimulated by a visual cue that had (automatically) attracted the animal's attention. Likewise, electrical stimulation of focal sites in the SC appears to direct the animal's attention to the corresponding site in the visual field. Surprisingly, however, we did not find enhanced responses from SC cells when the animal was instructed (cognitively) to attend to the stimulus in the absence of an explicit orienting cue. Thus, SC cells appear to form part of a circuit for automatically orienting attention to salient peripheral stimuli but are not involved in the cognitive, or "voluntary", control of attention. The results are explained within a new model of attentional control based on a distributed competitive network, and we will be testing this model over the next year.

3. Adaptive mnemonic filters in IT cortex. By gating V4 and IT neuronal responses, attention determines which visual information reaches the mechanisms for memory storage. Because little is known about how these storage mechanisms work at the level of individual cells, we have recorded from neurons in the most anterior portion of IT cortex of monkeys performing a working memory task. Most previous studies of memory in IT cortex have been limited to the standard delayed matching-to-sample paradigm, in which a sample stimulus is followed (after a short blank interval) by a test stimulus, and the monkey must indicate whether or not the test stimulus matches the sample. However, a useful neural mechanism for memory must have the capacity to retain information over relatively long intervals that are not "blank" but rather are filled with new stimuli entering the visual system, competing for processing.

To study neural mechanisms under realistic memory demands, we recorded from IT neurons in monkeys performing a mnemonic task that required them to actively retain items in memory while concurrently viewing new stimuli. We found that the responses of half the cells were suppressed according to how well the current stimulus matched the stimulus actively held in memory. Thus, IT neurons may function as adaptive "mnemonic filters." Temporal contiguity alone could not explain the results, as there was no modulation of responses when a stimulus on one trial was repeated on the next trial; an active reset mechanism appears to restrict the memory comparison to just the stimuli presented within a trial. Responses to stimuli that matched memory traces were suppressed from the earliest onset of the visual response and remained suppressed in a sustained fashion; we found no evidence for significant temporal modulation of the spike trains when we averaged responses over intervening stimuli. To determine how much mnemonic information was conveyed by individual IT cells, we analyzed the responses with discriminant analysis and neural network techniques. According to these techniques, one could achieve the same performance in the task as the animal by pooling

the responses of only 25 IT neurons. Thus, mnemonic information equivalent to that held by the animal as a whole is distributed down to the level of small neural populations.

We propose a model in which working memory is mediated by two populations of IT cells. One functions as adaptive mnemonic filters and the other functions as a sensory referent. The difference in response between the two populations is a measure of how far the current stimulus stands out from the stimuli of the recent past, a type of temporal figure-ground extraction.

Our results on working memory were all obtained with stimuli that were highly familiar to the monkey, and the modulation of responses we observed were confined to a single trial, lasting seconds, of the monkey's mnemonic task. However, when we began using novel stimuli in the task, i.e. stimuli that the monkey had never seen before, we observed longer-term influences on neural responses over the course of the hour-long recording session. As the novel stimuli gradually became familiar to the animal, we observed a gradual "focusing", or narrowing, of activity across the population of neurons. These effects were strikingly stimulus-specific, in that cells could detect that a particular stimulus had been seen before after more than a hundred intervening presentations of other stimuli. The results extend the adaptive mnemonic filter model to longer term memory traces, and suggest a role of IT neurons in recognition memory.

4. The role of acetylcholine. As a first step in exploring the pharmacology of the adaptive filter mechanism in IT, we tested the effects of scopolamine, a cholinergic antagonist, on the performance of both the animal and IT neurons in the working memory task described above. Although the animal's performance was impaired by scopolamine, we found little effect on IT neurons, suggesting that scopolamine impairs a mnemonic process "downstream" from IT. This surprising finding will be followed up over the course of the next year.

5. Extrastriate neurons and recalled memories. In our studies on working and recognition memory described above, animals matched the current stimulus to memory traces of physically identical stimuli presented in the immediate past. In many everyday situations, however, memory traces are actively "dredged up" through associative recall, either across or within sensory modalities. In recent work, we have found striking evidence for activation of specific extrastriate neurons during within-modality recall. In IT cortex, we found that when an animal is given a brief visual cue (at one spatial location) to search for an item (at a different location), neurons coding the searched-for item are activated until the item is found. When a given cue is used predictably for a block of trials, we have seen activation of specific IT neurons for more than fifteen minutes.

In area V4, we recently found that when the animal is instructed to hold its attention in a sustained fashion at one location in the visual field, neurons coding that specific spatial location are also activated for long periods in the absence of any stimulus - as long as attention is sustained. The level of sustained activation is often low, only a few spikes per second, but is very reliable.

In addition to the phenomenological experience of "recall", we believe that the function of sustained activation in these areas is to segregate the neurons that will provide the information needed for a given task. This segregation might be facilitated if the higher discharge rate led to synchronization of firing among the selected neurons, a possibility we are now preparing to test.

6. Visual discrimination learning and the caudal neostriatum. Although limbic damage or disease causes profound amnesia, certain learning capacities are spared, including the ability to learn new discrimination tasks. Behavioral studies in our lab and others suggest that the neostriatum plays an important role in mediating these spared capacities. Anatomical studies in our lab have shown that the tail of the caudate nucleus and the ventrocaudal putamen are the largest subcortical targets of the visual cortex, and they are also known to receive dopaminergic projections from the substantia nigra. However, the only previous neurophysiological study of the caudal neostriatum (the major visual portion) during performance of a visual discrimination task reported very poorly responsive cells that habituate rapidly. To resolve this puzzle, we began to record from the caudal neostriatum while monkeys learned new visual discriminations (in a go/no-go paradigm) as well as performed ones previously learned. Unlike the previous study, we found that neostriatal cells were both highly responsive and highly stimulus selective, resembling cells in the cortical areas that project to the neostriatum. However, preliminary data indicate that a small subpopulation of caudate cells changes its response during learning. As the animal learns, these cells appear to become more responsive to the no-go stimuli, i.e. the stimuli that do not elicit a behavioral response and are not rewarded. Clearly, we have just scratched the surface on the role of the neostriatum in learning and will be pursuing this 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. Furthermore, we have identified neural networks whose responses appear to

mediate both working and long-term memory. If, during the future course of this project, we can uncover some of the other critical components of attentional control and memory storage, 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, affective disorders, and schizophrenia.

PROPOSED COURSE OF RESEARCH:

The major thrust of our work over the next year will be to pursue the cortical mechanisms of attention and memory at the neuronal and, ultimately, synaptic level.

PUBLICATIONS:

Desimone, R. Neural circuits for visual attention in the primate brain. In G. Carpenter and S. Grossberg (Eds.): Neural Networks for Vision and Image Processing. MIT Press, Cambridge, pp. 343-364, 1992.

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Lehky, S.R., Sejnowski, T.J., and Desimone, R. Predicting responses of nonlinear neurons in monkey striate cortex to complex patterns. J. Neurosci., in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02037-11 LN

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*
Functional anatomy of the somatosensory cortex of monkeys

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.2	2.0	1.2

CHECK APPROPRIATE BOX(ES)

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

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

In a series of anatomical experiments, we have identified two routes by which somatosensory information reaches limbic structures important for somatic memory. One route courses dorsally from the postcentral strip through posterior parietal cortex and has access to the limbic system via cingulate cortex. This pathway may be functionally analogous to the occipitoparietal pathway for spatial vision. A second route courses ventrally from the postcentral strip through SII to the insular cortex and then to the amygdala and indirectly to the hippocampus through rhinal cortex. We now have evidence that this second pathway is analogous to the occipitotemporal pathway for object vision. Our neurobehavioral evidence indicates that bilateral insular lesions cause a severe tactile recognition deficit, consistent with the suggestion that insular cortex is a critical link in a parieto-insulo-limbic pathway for tactile recognition, and so occupies a position analogous to that of area TE in the occipito-temporo-limbic pathway for visual recognition. By recording neuronal activity from SII in hemispheres that received lesions of selected portions of the postcentral body representation, we demonstrated a loss of the corresponding representation from SII, indicating a serial flow of information from the postcentral strip to SII. An unexpected finding in this study was that, following a postcentral lesion, the deactivated SII region undergoes major functional reorganization. In another experiment we recorded neuronal activity from the postcentral strip in 4 monkeys 10-12 years after the dorsal roots representing the upper limb were severed. The results indicated that the entire representation of the upper limb had been replaced by an expanded face representation, an expansion an order of magnitude greater than previously thought possible. Both of these experiments, involving perturbations of the central nervous system, resulted in a previously unrecognized degree of cortical plasticity in adult mammals and suggest that the potential for reorganization is greater after central than peripheral nervous system manipulations.

PROJECT DESCRIPTION:

Previous work from our laboratory has shown that the amygdala and hippocampus are critical for both visual and tactual memory. We have been tracing the anatomical and functional routes by which tactual information could reach these limbic structures with the goal of determining whether there is a common cortical plan of organization across all of the sensory modalities for the processing, storage, and retrieval of sensory information. Further, to determine how visual and tactile information might be integrated, we have begun to examine the pattern of thalamic and cortical labelling following injections with anatomical tracers of both tactually and visually responsive regions of posterior parietal cortex and of the pulvinar nucleus of the thalamus. We have also begun to assess the capacity of both primary and nonprimary cortical maps to reorganize after central versus peripheral perturbations of the nervous system using electrophysiological and histochemical techniques. In addition we are studying the expression of various neurotransmitters at all levels of the somatosensory system in normal and operated animals to determine the mechanisms responsible for the reorganization seen in cortical maps. Finally, we have begun studies to examine whether fields of the motor cortex are organized in a parallel or hierarchical fashion and to identify the connections from sensory areas that are responsible for triggering movements.

Anatomical studies

Connections of somatosensory cortical areas. 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 perirhinal and 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, courses through posterior parietal cortex, and then projects to the cingulate cortex before finally reaching the amygdala and hippocampus.

To determine whether analogous pathways for stimulus quality and spatial perception might be present in all of the sensory systems, we turned to the somatosensory system, since more is known about its cortical organization than any other modality 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 cutaneous information, area 3b. Area 3b projects forward to area 1 and less densely to area 2. The densest cortical projection from each of these areas (3b, 1, and 2), however, is to layer IV of SII cortex. SII in turn 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. As indicated below, we have been slowly accumulating additional physiological and behavioral evidence that this pathway in the somatosensory system is analogous to the ventrally directed pathway in the visual system and consequently is important for the tactile identification of objects.

The second pathway, demonstrated partly from the studies described below, 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. Area 7b projects to the cingulate cortex and parahippocampal gyrus. Each of the above areas projects with a backward connection upon the cortical area from which it receives a forward connection. This dorsally projecting somatosensory pathway may be analogous to the occipitoparietal pathway in vision and thus could be important for spatial perception in somesthesis.

Traditionally, posterior parietal cortex has been thought of as "association" cortex, where information from different sensory modalities is integrated. To determine the anatomical basis for such integration we have undertaken studies in which we inject anatomical tracers into electrophysiologically defined portions of visually and tactually responsive regions of posterior

parietal cortex. Our findings indicate that visually and tactually responsive regions of posterior parietal cortex tend to have connections with other visually and tactually responsive cortical and thalamic regions, respectively. Thus our anatomical findings fit nicely with recent electrophysiological studies which suggest that much of posterior parietal cortex is modality specific, and therefore is not likely to serve as the site for multisensory integration or "association" of inputs from different sensory modalities. Interestingly, both visual and tactual posterior parietal cortex have rich interconnections with motor areas in the frontal lobe and receive their major thalamic input from the pulvinar complex, suggesting that posterior parietal cortex may serve a common function for both of these sensory modalities.

The pulvinar complex constitutes the largest nuclear mass of the primate thalamus, but at present we do not know the answers even to such basic questions as: 1) What are the major cortical projection targets of each of the four major subdivisions of the pulvinar? 2) What cortical and subcortical areas supply inputs to the pulvinar? 3) What are the relative contributions of cortical and subcortical inputs to the pulvinar? 4) Does the laminar projection to the cortex from the pulvinar obey the same rules as those from the principal sensory relay nuclei? In an attempt to answer these and other questions, we have placed injections of anatomical tracers into the four subdivisions of the pulvinar complex. Analysis of the cortico-pulvinar projections has revealed that the inferior and lateral pulvinar (Pi and Pl) receive inputs primarily from visual cortical areas, the anterior pulvinar (Pa) from somatosensory and motor cortical areas, and the medial pulvinar (Pm) from visual and motor cortex, somatosensory cortex, and, perhaps most densely, from multimodal cortical areas. These findings, like the connectional findings in posterior parietal cortex, indicate a similar organizational plan for processing information in touch and vision and also implicate the medial pulvinar in the mediation of associations between the tactual and visual systems. Additional analyses will be undertaken to assess the relative contribution of subcortical inputs to the pulvinar.

Connections of motor cortical areas. Presently there is some controversy in the literature as to whether motor cortical areas have a fine degree of somatotopic organization. The use of multiple anatomical dyes to trace neural pathways is a powerful tool to demonstrate the existence or absence of topography of neural connections. We have used such an approach to study the topography of sources of inputs to physiologically defined regions of MI. Thalamo-cortical projections include those from the oral ventral posterior lateral nucleus (VPLo) and the oral ventral lateral nucleus (VLo), with the facial and forelimb sectors of MI receiving input from the medial and lateral parts,

respectively, of both VPLo and VLo. The forelimb sector of MI is connected with lateral postarcuate cortex, whereas the facial sector is connected with adjacent, more lateral, parts of this cortex. Our findings indicate that a topographical pattern of connections with MI is more obvious in some cortical areas and thalamic nuclei than others.

While it is known that muscle afferent information reaches motor cortex, the pathway by which this is accomplished is not known. We have demonstrated, contrary to many earlier reports, that area 3a of somatosensory cortex provides a direct projection of afferent information to motor cortex. This cortical projection from 3a to motor cortex is a prime candidate for the pathway that supplies muscle afferent information to the cortical motor system, and is likely to be important for many aspects of sensorimotor integration. Interestingly, primary postcentral areas other than area 3a provide no (area 3b) or little (areas 1 and 2) input to area 4.

Histochemical Studies

Changes in the thalamus after ablations of the postcentral hand representations. Following ablation of all four hand representations in the postcentral strip, we see virtually complete retrograde degeneration of neurons in the corresponding portion of VP, the somatosensory thalamic relay nucleus to the cortex, but little, if any, such changes in SII. Likewise, there is a dramatic reduction in staining for a marker of cellular metabolism, cytochrome oxidase (CO), in VP but not in SII. We have now quantified these results and demonstrated virtual elimination of large projection neurons (>180 um^2) and a sharp decrease of small neurons (<160um^2) in the affected portion of VP. Most of the small neurons that do remain stain positive for the inhibitory neurotransmitter gamma aminobutyric acid (GABA) and are almost certainly interneurons instead of cortical projection neurons. Thus only a minor population of small sized VP neurons are left that could project to regions outside of the postcentral strip such as SII and the posterior parietal cortex, a finding which is consistent with our electrophysiological findings that 1) the thalamus is incapable of supplying somatic activation to SII in the absence of the postcentral strip, and 2) that all parts of SII continue to respond to somatic stimulation (see electrophysiological experiments below), even though a given body part representation has been deactivated.

The results obtained in the thalamus after partial removal of the postcentral cortical hand representations (e.g. 3b) differed from those obtained when complete ablations (extending across areas 3a, 3b, 1, and 2) were made. Instead of virtually complete degeneration of large projection neurons throughout the hand representation in VP, as was the case after complete ablations of

the hand representations, after partial removals we found alternating regions in the hand representation of VP that appeared qualitatively normal or completely degenerated. Similarly, CO staining revealed dark and light patches of thalamic label that corresponded precisely to the normal and degenerated thalamic regions. Although the dark CO patches in VP appeared qualitatively to be stained as intensely as in the normal thalamus, such was not the case when examined quantitatively. This prompted us to perform cell counts in the dark patches and compare them with cell counts from identical areas in control animals. The result of this analysis showed a statistically significant reduction in the number of large projection neurons in the dark patches. This finding demonstrates that although portions of VP may project differentially upon the cortical areas comprising the postcentral strip, all portions of the hand representation in VP have at least limited direct connections to all four of these cortical areas. This contradicts recent reports that areas 3a, 3b, 1, and 2 each receive projections from separate portions of VP.

Since some small neurons that do not react positive for GABA remain in the hand portion of VP after complete removal of the postcentral hand representations, the question arises as to what function these surviving neurons may be serving. Recently, E.G. Jones and his colleagues at Irvine have identified a compartmental organization of VP with alternating zones rich in parvalbumin and calbindin. The parvalbumin positive domain receives its input from the dorsal column nuclei and projects to middle cortical layers of postcentral cortex. By contrast, the calbindin positive domain receives its input from the spinothalamic tract and terminates in superficial layers of postcentral cortex. The parvalbumin domain in VP consists mostly of large thalamic cells, which degenerate completely after postcentral cortical ablations, whereas only small neurons are normally present in the calbindin domain of VP. We are presently collaborating with Jones to determine whether the population of small non-GABA neurons that survive in VP after postcentral cortical ablations are calbindin positive. If so, such calbindin positive neurons would provide evidence for a widespread thalamocortical projection system to superficial layers of a large number of cortical areas.

Correlations between glutamate-immunoreactivity and somatotopic reorganization. We and others have now demonstrated that the adult primate brain possesses a remarkable capacity for changes in cortical maps of sensory receptor sheets in response to experimental perturbations of peripheral or central sensory inputs. The mechanisms responsible for such reorganizational changes, however, still remain a mystery. Since the expression of neurotransmitters and neuromodulators, as well as of postsynaptic molecular structures, is activity dependent,

post-injury changes in neurotransmitter levels could provide a mechanism for the type of reorganizational changes we have seen. To examine this possibility we have reacted sections to determine if there are increases or decreases in various neurotransmitters at different processing stations in the monkey somatosensory system 8 weeks after cutting and tieing all three nerves innervating the hand. Of the several different neurotransmitters we have studied so far, changes were found only in glutamate, indicating that this neurotransmitter could play a major role in the regulation of adult neural plasticity.

We have now studied the pattern of glutamate-immunoreactivity (Glu-ir) in four monkeys with complete denervation of the hand. The pattern of GLU-ir in the dorsal horn of the spinal cord, in the dorsal column nuclei, and in the ventroposterior nucleus of the thalamus, showed no obvious difference from that of normal animals. Similarly, Glu-ir neurons in each of the cytoarchitectonic areas comprising the postcentral strip were regularly distributed and their morphology and laminar distribution were the same as in normal animals. In contrast, SII cortex displayed regions of normal Glu-ir alternating with regions in which Glu-ir was either absent or dramatically reduced. The Glu-ir affected regions were located in the hand representation in SII and corresponded to cytochrome oxidase poor regions seen in adjacent sections. Examination of Nissl staining of the same regions in adjacent sections revealed no obvious changes in cytoarchitecture. A quite different result was obtained when examining SII of two monkeys that had complete ablation of the hand representations in the postcentral strip. In these animals there was no reduction of Glu-ir or cytochrome oxidase staining in any portion of SII.

Each of the monkeys used in the above study had undergone electrophysiological mapping of cortex normally representing the hand in both the postcentral strip and SII (see below). The results of these mapping experiments revealed that in animals that received nerve cuts, the postcentral hand representation had already begun substantial reorganization, whereas the SII hand representation was still unresponsive to somatic stimulation and thus had not reorganized. By contrast, in animals with complete ablation of the postcentral hand representations, recordings in SII revealed that the former hand representation had become completely reorganized to represent the foot.

Taken together, the electrophysiological and immunohistochemical experiments suggest that reduction in glutamate levels may be one mechanism contributing to the overall reduction or elimination of neural activity in the SII hand representation after the hand is completely deafferented and that the presence of glutamate is necessary for reorganization to occur. The evidece is of course only correlative at this point. Future experiments will be

directed toward determining whether the reduction in glutamate causes the failure of cortical reorganization or vice versa. Finally, in view of the well established involvement of the NMDA-type of excitatory amino acid receptor in use-dependent developmental plasticity, our findings suggest that NMDA receptors might also play an important role in the reorganizational processes that occur in the adult cerebral cortex after perturbations of either the peripheral or central nervous system.

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 200um 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 postsurgical 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 body-part 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.

Of the four areas that comprise postcentral somatosensory cortex, two (areas 3b and 1) process predominantly cutaneous inputs, whereas the other two (areas 3a and 2) process mainly "deep" somatic inputs. We made selective removals of the hand representations in areas 3a, 3b, 1 and 2 in different combinations to determine each area's contribution to the responsivity and modality characteristics of neurons in SII. Ablations that left intact one or both postcentral areas that process "deep" inputs yielded SII recording sites mainly responsive to "deep" stimulation. Conversely, removals that left intact one or both postcentral areas that process predominantly cutaneous information yielded SII recording sites responsive to cutaneous stimulation, and none that was driven exclusively by "deep" stimulation. When only the area 3b hand representation was ablated, leaving areas 3a, 1, and 2 fully intact, there was a sharp reduction (though not elimination) in the number of cutaneous recording sites in the SII hand representation, but not in the number of "deep" sites. The ratio of cutaneous and "deep" sites for body part representations other than the hand was not affected by any of the ablations, indicating that the above results were not due to a general postsurgical depression or to anesthetic effects. These findings suggest that modality-specific information is relayed from postcentral cortex to SII along parallel channels, with cutaneous inputs being transmitted via areas 3b and to a lesser extent area 1, and "deep" inputs via areas 3a and 2. Further, we have demonstrated that area 3b provides the major source of cutaneous inputs to SII, either directly or via area 1. Finally, these recording experiments demonstrate that somatic activation of neurons in SII is achieved by both serial and parallel cortical processing.

Lesion-induced cortical plasticity. As indicated above, an unexpected result of our original electrophysiological work was the finding that SII undergoes major functional reorganization following total removal of the hand representations in postcentral cortex. Although initially the affected region in SII is unresponsive, later the 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. Indeed, the areal extent of the foot representation increases to occupy most of the former hand region (a distance of 5 or more millimeters of cortex). Interestingly, the partial ablation studies described above indicated that leaving even a portion of the hand representation intact in any of the postcentral areas is sufficient to block the expansion of the foot representation in SII.

It has often been suggested that reorganization in primary somatosensory cortex after peripheral nerve manipulations is due to changes at the cortical, as opposed to the subcortical level, though there has been no direct evidence for this proposal. The findings on SII plasticity just presented, taken in conjunction with our previous finding that precentral cortex is the source of somatic activation of SII, provides the first direct evidence for cortically mediated reorganization in adult mammals. Further, they provide evidence for a previously unrecognized degree of cortical plasticity in adults and raise the possibility that nonprimary cortical areas have a greater capacity for reorganizational changes than do primary cortical areas. Another possibility, which does not exclude the first, is that perturbations of the central nervous system result in greater reorganizational changes than perturbations of the peripheral nervous system.

Cortical plasticity after peripheral deafferentation. To compare the effects of central versus peripheral deafferentation on somatotopic reorganization in both primary and nonprimary somatosensory cortex, we cut and tied the three nerves normally innervating the hand and, six to eight weeks later, recorded from both primary (postcentral strip) and nonprimary (SII) cortex. We found that cortex normally devoted to the postcentral hand representations had become responsive to somatic stimulation of both the arm and face, although stimulation thresholds for eliciting a neuronal response were abnormally high. By contrast, sites in the expected location of the SII hand representation were largely unresponsive to somatic stimulation. The few sites in this region that did respond had receptive field locations and response thresholds that were virtually identical to those in the reorganized portion of the postcentral strip. The presence of receptive fields on the face for recording sites several millimeters more medial than the normal location of the face representation in the postcentral strip constituted a surprising expansion of this representation.

To determine if such an expansion would be blocked if the nerve innervating the portion of the hand map immediately adjacent to the face map were allowed to regenerate, we cut the three nerves innervating the hand but this time allowed regeneration of the

median nerve, which normally supplies input to the region of cortex immediately next to the face map. Our findings indicated that median regeneration was sufficient to block the medial expansion of the face representation and, additionally, that the median nerve field itself expanded medially.

Since both the face and median hand representations expanded from lateral to medial across the postcentral strip, we next asked whether medial to lateral expansion also was possible. To address this issue we cut the three nerves to the hand but allowed the ulnar nerve to regenerate. The results revealed not a lateral expansion of the ulnar field but, instead, a medial expansion of the face representation. Thus, cortex normally representing the hand does not prefer other hand inputs over face inputs when reorganizing. Rather, for unknown reasons, reorganization in the postcentral strip appears to follow a lateral to medial gradient.

As already indicated, there was little or no reorganization in SII 6-8 weeks after complete deafferentation of the hand by peripheral nerve cuts. The explanation for this failure, given reorganization in the postcentral strip after the same nerve sections, and reorganization in SII after postcentral ablations, is unclear. One possibility is that, since SII is dependent upon postcentral cortex for its activation, full reorganization and activation of postcentral cortex must occur before SII can become reorganized.

The differences in the magnitude of cortical reorganization seen after peripheral versus central nervous system perturbations suggest that peripheral manipulations result in minimal reorganizational changes within the cortex itself. Perhaps this is because changes that take place early in an ascending system are simply relayed to the next processing station along "hard wired" connections, imposing constraints on the changes that could take place at this next level. Conversely, central manipulations, such as ablation of the postcentral hand representations, directly remove axonal terminals on cells in the next processing station, opening up a relatively large synaptic space for inputs from other sources. As described below, we recently determined whether a central manipulation at the closest possible point to the peripheral nervous system would result in more extensive reorganization than peripheral nerve manipulations.

Plasticity in the postcentral strip following long-standing dorsal rhizotomies. In a dorsal rhizotomy, sensory nerve roots are severed at a point where they exit the dorsal root ganglion, and are thus a central nervous system manipulation. Such a procedure denervates ascending sensory information to the brain stem, thalamus, and cortex. Thus the cortical sensory maps that

normally receive inputs from the deafferented region might be expected to simply remain deactivated, i.e. unresponsive to somatic stimulation. We recently had the opportunity to record electrophysiological responses from the postcentral cortex of four monkeys that, 10-12 years earlier, had undergone unilateral or bilateral dorsal rhizotomies of the nerve segments representing the upper limb. The results indicate that this entire upper limb representation had become completely and totally reorganized. These unexpected findings extend the previously presumed upper limit for cortical reorganization by an order of magnitude (i.e. 1 cm^2, or 1/3 of the postcentral strip) and raise the possibility that the upper limit may be even greater! Further, because this degree of reorganization greatly exceeds the projections zones of single axons from the thalamus, the findings point to some subcortical structure as the likely locus of the changes.

Receptive fields of insular neurons. In another recording project, we are mapping the somatically responsive portions of the insular cortex, which our anatomy shows 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 is being performed in awake monkeys trained to sit quietly in a primate chair and to allow gentle tactile stimulation of their bodies. Preliminary results indicate that receptive fields of posterior insular neurons are selectively tactile, bilateral, and very large.

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 visuo-limbic pathway.

Microstimulation studies of motor cortex. The recovery of motor function known to occur following damage to primary motor cortex, MI, has traditionally been attributed to subcortical reorganization. Further, most current theories on the cortical control of movement hold that "premotor" (area 6) and "supplementary" motor cortex (SMA) act to program primary motor cortex (MI) and that MI corticospinal projections are normally responsible for the cortical control of movement. However, motor areas outside of MI also have corticospinal projections, raising the possibility that the cortical control of movement is mediated

by multiple corticospinal pathways organized in parallel. To examine this hypothesis directly, we have begun experiments using microstimulation to identify and map the primary and supplementary areas of motor cortex in the relatively agyric owl monkey. After extensively mapping various movement representations and determining current thresholds for each site, we ablated either the MI hand or vibrissa representation and then remapped the SMA and vice versa. Our findings indicate that MI and SMA are each sufficient to drive movements in the absence of the other and that stimulation thresholds are unaffected, providing strong support for the proposal that movement is under the control of at least two parallel corticospinal systems.

Neurobehavioral studies

We have recently completed a study examining the behavioral effects of bilateral lesions of insular cortex and have postoperative results indicating that such lesions cause a tactile, but not a visual, recognition deficit. A comparison group with bilateral area TE lesions showed the opposite effects, thereby yielding a double dissociation of deficits. This finding is consistent with our hypothesis that insular cortex acts as a final link in a parieto-insulo-limbic pathway for tactile memory, analogous to the occipito-temporo-limbic pathway for visual memory. These findings demonstrate that cortical processing of sensory and mnemonic information shares a common plan for the somatosensory and visual modalities and perhaps other sensory modalities as well.

Local cerebral blood flow and oxygenation studies

We have begun collaborating with investigators at the NIH in vivo MRI center to localize and measure changes in cerebral blood flow or blood oxygenation that are correlated with neural activity. In the anesthetized monkey we were able to detect changes in blood flow to the brain induced by increasing CO_2 levels. Our measurements indicate blood flow changes on the order of 30%, which agree well with other apnea studies. We have also begun examining changes in blood oxygenation to visual, tactile, and motoric stimulation. Presently, using the 4.0 Tesla magnet, we are able to measure changes of up to 16% in awake humans either exposed to intense visual stimuli or making hand movements. The technique is close to being fully operational and we hope soon to start a series of experimental studies to identify regions of the brain involved in various perceptual and cognitive functions in humans, based on our nonhuman primate models.

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. In addition, the studies have suggested remarkable parallels between the organization of the somatosensory and visual systems and imply that similar mechanisms of cortical processing for perception and memory may operate within both. These projects are thus 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. Further, we have demonstrated a previously unrecognized degree of post-injury plasticity in the adult macaque with the exciting discoveries that the postcentral strip undergoes massive functional reorganization following dorsal rhizotomy and SII does so following damage to area SI. By comparing differences in somatotopic reorganization in different cortical areas and after various central versus peripheral perturbations, we suggest that sensory cortex may have a much greater capacity for reorganization after central damage than after peripheral damage. The results have far reaching implications regarding the potential for functional recovery following damage to the nervous system.

PROPOSED COURSE OF RESEARCH:

More neurobiological and behavioral evidence is required to assess our hypothesis that the ventrally directed cortical pathway in the somatosensory system is analogous to the ventrally directed one in the visual system, and the necessary studies will be continued. In addition, we plan to begin research projects designed to determine if the dorsally directed somatic cortical pathway has a role in tactual spatial perception by analogy to the role of the dorsal visual pathway in visual spatial perception. We have begun studies that attempt to identify the mechanisms responsible for the remarkable post-injury reorganizational changes we have observed in the adult brain and also to determine whether such reorganization has any adaptive perceptual or cognitive correlates or whether, instead, it is actually detrimental to normal function. In addition we have begun studies in the MRI center with the goal of activating tactile cortical areas and identifying reorganized cortex in humans who have suffered limb amputations or paralysis due to stroke. Finally, we are beginning to explore how the motor system is organized, with the ultimate goal of understanding how sensory processes trigger and guide movements.

PUBLICATION:

Pons, T.P., Garraghty, P.E., and Mishkin, M. Serial and parallel processing of tactual information in somatosensory cortex of rhesus monkeys. J. Neurophysiol., in press.

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PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*
Ontogenetic development and decline of cognitive memory and habit formation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	M.J. Webster	Visiting Associate	LN NIMH
Others:	M. Mishkin	Chief	LN NIMH
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	L.G. Ungerleider	Research Psychologist	LN NIMH
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COOPERATING UNITS *(if any)*

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SECTION

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.3	2.0	3.3

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced 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-striatal system, respectively. To see how emotional and social behavior develop in the absence of cognitive memory (i.e. in the presence of a global amnesia that persists from infancy through adulthood) we 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. Interestingly, although early damage to the limbic memory system did not yield the Kluver-Bucy symptoms of loss of fear, indiscriminate approach to objects, and coprophagy, seen in monkeys given limbic lesions in adulthood, it produced other behavioral abnormalities, such as lack of social interactions, blank facial expressions, and motor stereotypies. The developmental time course and the nature of these disturbances resemble those seen in autistic children. Assessment of the effects of the neonatal lesions on the early development of habits and memories points to greater compensatory potential after neonatal cortical than after neonatal limbic removals, suggesting that cortical association areas are relatively immature at birth. 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. Data on both normal and operated infants suggest that development of the cortico-neostriatal habit system is sexually dimorphic, and that this is due to the high testosterone levels present in male infants before and shortly after birth. Finally, 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 the gradual decline in memory ability that occurs with normal aging.

PROJECT DESCRIPTION:

Our studies in adults have suggested that cognitive memory and habit formation are two qualitatively different learning processes based on a cortico-limbic and a cortico-striatal system, respectively. Our initial studies in infant monkeys indicated that these two systems are developmentally dissociable, with the habit system maturing earlier than the cognitive memory system. A similar delay in the maturation of the limbic memory system has been demonstrated recently in human infants. The conclusion that the habit system matures early must be qualified as follows. First, whereas 3-month-old female infant monkeys can form a set of visual discrimination habits as quickly as adults, 3-month-old male infants are significantly though only temporarily retarded; also, neonatal ablation of area TE impairs the learning of female but not of male infants, even though the sexes are equally impaired when the lesions are made in adulthood. Both findings suggest that ontogenetic development of the habit system is sexually dimorphic, this system maturing earlier in females than in males. This sexual dimorphism seems to be dependent on the high testosterone levels found in male infants before and shortly after birth, because (1) there is a significant correlation between their testosterone levels and learning scores (the higher the testosterone level, the slower the learning), and (2) orchiectomy in male infants speeds their rate of habit formation, whereas dihydrotestosterone in ovariectomized females slows their rate. The conclusion that the cognitive memory system matures late also must be qualified. Although visual recognition memory measured by problem-solving tasks does indeed develop late, it can be demonstrated in early infancy when measured by a preferential-viewing task. This primitive recognition memory is nevertheless markedly impaired by neonatal limbic-system damage, though not by neonatal ablation of inferior temporal cortical area TE.

With regard to the late formation of cognitive memories as measured by problem solving (i.e. visual delayed nonmatching-to-sample or DNMS), monkeys with neonatal limbic (i.e. amygdalo-hippocampal) lesions were severely impaired both at 10 months and 5-6 years of age, whereas those with neonatal TE lesions showed substantial functional sparing at both ages (compared to adults given TE lesions). In addition, at 5-6 years of age, the monkeys with neonatal limbic lesions were also impaired in tactile and spatial DNMS, indicating that early damage to limbic structures results in a permanent, global, sensory memory loss. To determine the separate contributions of the amygdaloid complex and hippocampal formation to the development of cognitive memories, we evaluated the effects of selective neonatal lesions of either the amygdaloid complex and rostral entorhinal cortex (A) or the hippocampal formation, caudal entorhinal cortex, and parahippocampal gyrus (H) on

recognition memory. At 10 months of age, monkeys with neonatal H lesions were not impaired, whereas those with neonatal A lesions were significantly impaired, though their impairment was not as severe as that of monkeys with the combined lesions (AH). Thus, in the monkey, either the amygdala, the rostral entorhinal cortex, or both contribute more than the hippocampal formation to the development of recognition memory.

The long-term effects of these early lesions indicate that compensatory mechanisms operate early to promote permanent recovery from neonatal area TE lesions but not from neonatal limbic lesions. These findings 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 is provided by our neurobiological studies showing that the distribution of both cholinergic and opiatergic receptors is adult-like at birth in subcortical structures and allocortical areas but is not yet fully developed in neocortical areas, particularly the sensory association cortex, and that adult levels of metabolic activity in visual association cortex and particularly area TE are not reached until about 6 months of age. This evidence suggests that the relatively poor recognition ability of normal neonates (and perhaps, by extension, the phenomenon of infantile global amnesia, i.e. the inability to recall any of the stimuli or events experienced in infancy) is more likely due to slow maturation of the cortical association areas than to neonatal immaturity of the limbic system. Direct evidence of neocortical plasticity in the infant macaque has been provided by our recent anatomical and behavioral studies showing that (a) there exist transient projections from area TEO to the amygdala and to the perirhinal areas in infant monkeys which retract during maturation, (b) the transient projections to the amygdala become stabilized in infants with neonatal removals of area TE, and (c) area TEO as well as cortical areas (such as PG, TF, and STP) normally connected to limbic structures but not normally involved in object recognition assume a critical role in this memory function when area TE has been removed in early infancy.

We have also investigated the socio-emotional behavior of the infants with neonatal lesions. Early damage to limbic structures does not yield the Kluver-Bucy symptoms of loss of fear, indiscriminate approach to objects, and coprophagy, seen in monkeys given limbic lesions in adulthood. Nonetheless, compared with intact infant monkeys and those with neonatal cortical lesions, monkeys with neonatal limbic lesions begin to show numerous socio-emotional abnormalities as they mature (e.g. lack of social interactions, blank facial expressions, and motor sterotypies). These abnormalities began to appear when the animals were 6 months of age and were still present at 6-7 years of age, indicating that early damage to the limbic system yields

long-lasting socio-emotional disturbances. The developmental time-course and the nature of the disturbances in these animals resemble those seen in autistic children. Our developmental studies together with the recent report by Kemper et al. documenting neuropathology in the amygdala, hippocampus, and cerebellum of the brains of five autistic subjects, provide evidence that early dysfunction of the limbic system may be one cause of infantile autism. Interestingly, although the operated controls that had received early damage to area TE showed none of the socio-emotional disturbances seen in infant monkeys with early limbic lesions, they did display other behavioral abnormalities (e.g. hyperactivity, and increased frequency in shifting behavior activities) that resemble those of children with an attention-deficit hyperactivity disorder. These behavioral abnormalities in monkeys with early damage to area TE were much less apparent when the animals reached adulthood (6-7 years). In addition, recent studies indicate that early damage to the hippocampal formation alone yields behavioral disturbances that appear only very late in development. The developmental time course and nature of these disturbances are somewhat reminiscent of those seen in schizophrenia.

Our studies in normal aged monkeys (performed in collaboration with investigators from the Johns Hopkins School of Medicine) indicated a gradual decline in learning and memory abilities with normal aging: (a) the behavioral decline appears to begin in the late teens for certain spatial abilities but does not affect other abilities (e.g. cognitive memory and habit formation) until the late 20s, suggesting that although the cerebral dysfunction eventually becomes widespread, the cerebral systems underlying spatial abilities are compromised by aging earlier than others; (b) the learning and memory impairments varied widely from one aged animal to another within a given task, suggesting that different animals have different patterns of cerebral dysfunction. This possibility is currently being investigated directly through post mortem localization of neuritic plaques and depletion of cholinergic and other neurotransmitter activity as a function of age.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

This project will provide the first comprehensive evaluation of the cognitive and social development of monkeys suffering from an amnesia induced by limbic lesions early in infancy as compared to those rendered amnesic in adulthood. It will help us understand those errors of central nervous system maturation that cause children to become autistic, dyslexic, learning disabled, or mentally retarded. Our neurobiological studies indicate how brain maturation normally progresses postnatally, and our neuroendocrinological studies demonstrate how the perinatal hormonal environment may influence brain maturation and,

consequently, the development of cognitive functions. Our studies on brain plasticity are yielding new insights into the neural mechanisms underlying sparing of function following early brain injury. Finally, the studies in normal aged animals are providing the anatomical and chemical basis for understanding the memory disorders in humans that accompany normal aging.

PROPOSED COURSE OF RESEARCH:

Our goal is to continue examination of the long-term effects of neonatal limbic lesions on social-emotional behavior and on learning and memory in order to test whether such a preparation does indeed provide an animal model of childhood autism. We shall also pursue studies to determine how recognition memory measured by preferential viewing differs from recognition memory measured by problem solving and compare the developmental time-course of this ability in infant monkeys to that seen in human infants. 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 early infancy in the absence of area TE, the highest-order area of the visual pathway.

PUBLICATIONS:

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Bachevalier, J. and Merjanian, P.M. The contribution of medial temporal lobe structures in infantile autism: A neurobehavioral study in primates. In M.L. Bauman and T. Kemper (Eds.): Innovation in Autism. Johns Hopkins Press, Baltimore, in press.

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Hagger, C. and Bachevalier, J. Visual habit formation in 3-month-old monkeys (Macaca mulatta): reversal of sex difference following neonatal manipulation of androgens. Behav. Brain Res. 45: 57-63, 1991.

Overman, W.H., Bachevalier, J., Turner, M., and Peuster, A. Object recognition vs object discrimination: comparisons between human infants and infant monkeys. Behav. Neurosci. 106: 15-29, 1992.

Webster, M., Ungerleider, L, and Bachevalier, J. Lesions of inferior temporal area TE in infant monkeys alter cortico-amygdalar projections. NeuroReport 2: 769-772, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02039-10 LN

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*
Pharmacology of Cognitive Memory and Habit Formation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	T.G. Aigner	Research Pharmacologist	LN NIMH
Others:	M. Mishkin	Chief	LN NIMH
	S. McBride	Psychologist	LN NIMH
	S. Weiss	Sr. Staff Fellow	BPB NIMH
	R. Post	Chief	BPB NIMH
	K. Bankiewicz	Visiting Scientist	SNB NINDS

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Neuropsychology

SECTION

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (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 suggests that the basal forebrain cholinergic system is critical for normal mnemonic function. In support of this proposal, we have found that visual recognition memory in macaques is impaired following either excitotoxic lesions of this system or administration in normal monkeys of the muscarinic receptor antagonist scopolamine. Furthermore, our studies indicate that scopolamine affects other forms of memory besides recognition, such as object-reward association and spatial memory, though the latter to a lesser degree. In addition, we found that scopolamine affects primary as well as secondary memory, and memory storage, not retrieval. On a computer-automated memory test, we have shown that the cholinesterase inhibitors physostigmine, THA, and E2020 all produce significant, though small, improvements in performance. Conversely, the NMDA receptor antagonist MK-801 impairs recognition memory, and it does so to the same degree as scopolamine, although MK-801 also produces an increase in response bias, suggesting the two drugs act via different mechanisms. Using in vivo microdialysis in anesthetized monkeys, we have shown that THC increases acetylcholine release in the hippocampus on the first but not subsequent administrations, indicating that some form of acute tolerance had developed. Our microdialysis studies in awake monkeys indicate that acetylcholine levels increase when an animal is behaving compared to when it is sitting quietly.

PROJECT DESCRIPTION:

Experiment 1:

There is now a large body of evidence that the cholinergic system plays a critical role in processes of learning and memory. We have previously shown in rhesus monkeys that the cholinergic drugs physostigmine and scopolamine produce dose-related improvements and impairments, respectively, in the number of objects correctly remembered in a delayed nonmatching-to-sample (DNMS) task. More recently, to test the suggestion that the N-methyl-D-aspartate (NMDA) receptor also participates in processes related to learning and memory, we compared the effects of physostigmine and scopolamine with those of the NMDA receptor antagonist MK-801 in monkeys performing a computer-automated version of DNMS. Physostigmine (10 μg/kg) significantly improved the percentage of correct DNMS choices, whereas scopolamine and MK-801 (32 μg/kg) produced a significant impairment compared to control performance under saline. We found in addition that scopolamine (32 μg/kg), but not physostigmine or MK-801, significantly increased response latencies for both sample and choice; MK-801 (32 μg/kg), but not physostigmine or scopolamine, significantly increased response bias (Index Y); and physostigmine (10 μg/kg) significantly improved DNMS performance without affecting response latency or response bias. Although both scopolamine and MK-801 impaired DNMS performance similarly, the dissociation of their effects on response latency and response bias suggests they did so through different mechanisms of action.

Experiment 2:

We have continued to use in vivo microdialysis in awake and anesthetized monkeys to study neurotransmitter levels under a variety of conditions. For example, we have shown that acetylcholine levels in the hippocampus increase when an animal is actively performing a task in the automated testing apparatus compared to when it is sitting quietly. We have also shown that peripheral injection of the muscarinic antagonist scopolamine causes an initial 200% increase in acetylcholine levels in the hippocampus and frontal cortical areas in the anesthetized monkey. In collaboration with Krzysztof Bankiewicz (NINDS), we have used the microdialysis technique to study dopamine-acetylcholine interactions in the striatum of monkeys and rats made hemiparkinsonian by unilateral injection of 6-hydroxydopamine. After the latter treatment, dopamine levels on the lesioned side were reduced by more than 90%, whereas acetylcholine levels on this same side were increased by approximately 80%. Amphetamine perfusion more than doubled dopamine levels in the caudate nucleus on the intact side, but not on the lesioned side; acetylcholine levels, on the other

hand, increased by approximately 70% on both sides, suggesting that acetylcholine changes are mediated by a dopaminergic-independent mechanism.

Experiment 3:

In collaboration with Susan Weiss and Robert Post of the Biological Psychiatry Branch, we have continued our investigations into the mechanisms underlying the reinforcing effects of cocaine and possible methods to antagonize these effects. We have shown that orally-administered carbamazepine significantly reduces the amount of cocaine intravenously self-administered by monkeys. We are pursuing these findings in three ways. First, we are completing negotiations on a Cooperative Research And Development Agreement with Pharmavene, Inc. that will allow us to study novel formulations of carbamazepine and other putative cocaine antagonists in our self-administration paradigm. Second, we are using our microdialysis-HPLC techniques to measure cocaine and neurotransmitter (e.g. dopamine) levels in selected brain areas in monkeys that are self-administering cocaine. Third, in collaboration with Barry Richmond in our lab, we are applying electrophysiological recording methods to determine if neuronal firing patterns in areas such as the nucleus accumbens can be related to cocaine self-administration behavior. (See Project Report Z01 MH 02032-15 LN.)

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Our results continue to provide convincing evidence that cholinergic mechanisms are essential to cognitive processes in monkeys. Our expanding use of in vivo dialysis in awake and anesthetized animals will provide new insights into the complex behavioral and biochemical interactions of acetylcholine and other neurotransmitters, as well as the mechanism of action of drugs of abuse such as cocaine.

PROPOSED COURSE OF RESEARCH:

We will continue to study the effects on cognitive memory and habit formation of peripheral and intracerebral injections of compounds that act via the neurotransmitter systems. We will continue to use microdialysis to study the basal forebrain cholinergic and the nigrostriatal dopaminergic systems in cognitive memory and habit, respectively. We will use these and other methods, such as electrophysiological recording to study the neuronal mechanisms underlying the reinforcing effects of cocaine and other drugs of abuse.

PUBLICATIONS:

Aigner, T. and Mishkin, M. Scopolamine impairs recall of one-trial stimulus-reward association in rhesus monkeys. Behav. Brain Res., in press.

Aigner, T., Mitchell, S., Aggleton, J., DeLong, M., Struble, R., Price, D., Wenk, G., Pettigrew, K., and Mishkin, M. Transient impairment of recognition memory following ibotenic-acid lesions of the basal forebrain in macaques. Exp. Brain Res. 86: 18-26, 1991.

Aigner, T., Saunders, R., Chavoix, C., and Frank, J. Application of magnetic resonance imaging to a primate model of the cholinergic deficit of Alzheimer's disease. In T. Nagatsu (Ed.): Basic, Clinical, and Therapeutic Aspects of Alzheimer's and Parkinson's Diseases. Plenum Press, New York, pp. 63-66, 1990.

Doudet, D., McLellan, C., Aigner, T., Wyatt, R., Adams, H., Miyake, H., Finn, R., and Cohen, R. Post-injection L-phenylalanine increases basal ganglia contrast in PET scans of 6-^{18}F-DOPA. J. Nuclear Med. 32: 1408-1413, 1991.

Doudet, D., McLellan, C., Aigner, T., Wyatt, R., and Cohen, R. Improved evaluation of specific to nonspecific 18F-DOPA uptake in brain. Ann. Neurol., in press.

Doudet, D., McLellan, C., Carson, R., Adams, H., Miyake, H., Aigner, T., Finn, R., and Cohen, R. Distribution and kinetics of 3-O-methyl-6- [18F]fluoro-L-DOPA in the rhesus monkey brain. J. Cereb. Blood Flow Metab. 11: 726-734, 1991.

Doudet, D., Miyake, H., Suddath, R., Aigner, T., Wyatt, R., and Cohen, R. Striatal presynaptic dopaminergic function and cerebral blood flow in the MPTP model of Parkinson's disease. In T. Nagatsu (Ed.): Basic, Clinical, and Therapeutic aspects of Alzheimer's and Parkinson's disease. Plenum Press, New York, pp. 87-90, 1990.

McLellan, C., Doudet, D., Brucke, T., Aigner, T., and Cohen, R. New rapid analysis method demonstrates differences in 6-18F-L-DOPA plasma input curves with and without carbidopa and in hemi-MPTP lesioned monkeys. Int. J. Radiat. Appl. Instr. 42: 847-854, 1991.

Post, R., Weiss, S., and Aigner, T. Carbamazepine in the treatment of cocaine abuse. In S. Korenman and J. Barchas (Eds.): Biological Basis of Addiction. Oxford University Press, Oxford, in press.

Saunders, R.C., Aigner, T.G., and Frank, J.A. Magnetic resonance

imaging of the rhesus monkey brain: use for stereotactic neurosurgery. Exp. Brain Res., in press.

Wang, J., Skirboll, S., Aigner, T., Saunders, R., and Bankiewicz, K. Methodology of microdialysis in the neostriatum of hemiparkinsonian nonhuman primates. Exp. Neurol. 110: 181-186, 1990.

Weiss, S., Post, R., and Aigner, T. Potential utility of the anticonvulsant carbamazepine in the treatment of cocaine abuse disorders: Clinical and mechanistic implications. In R. Watson (Ed.): Alcohol and Drug Abuse Reviews, Vol. 3. Humana Press, New Jersey, in press.

PATENT:

Weiss, S., Post, R., and Aigner, T.: U.S. Patent 4,942,182: Treatment for cocaine addiction, July 17, 1990.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02619-01 LN

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the*
Neural mechanisms of motivation and reward

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal*

PI:	B.J. Richmond	Captain	LN NIMH
Others:	Thomas Aigner	Research Pharmacologist	LN NIMH
	Eric Bowman	IRTA Fellow	LN NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Neuropsychology

SECTION

INSTITUTE AND LOCATION
NIMH, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

To quantify motivational state in monkeys, we have developed a behavioral paradigm in which monkeys are faced with the same task demands from trial to trial but with different expectancies regarding the amount of work needed to earn a juice reward. On each trial, the monkey is required to respond manually to a change in the color of a visual stimulus in the center of a video monitor. A modified variable-ratio reinforcement schedule is used in which progress toward earning a reward is indicated by a second visual cue located above the color stimulus. The monkey's accuracy and latency improve dramatically as the time of reward gets closer. Since the behavioral demands remain constant, the improvement in performance appears to reflect increased motivation. When a monkey was given intravenous cocaine for reward instead of juice, the effect of cueing the animal's progress toward earning a reward was disrupted. This paradigm will be used in studies directed at relating the activity of neurons in the ventral striatum and associated structures to levels of motivation for juice or cocaine reward.

PROJECT DESCRIPTION:

Objectives:

Although the molecular biology, pharmacology, and anatomy of the neural structures thought to underly motivation have been the subjects of intense study, their integrative functions are poorly understood. For example, it is known that neurotoxic lesions or pharmacological blockade in the ventral striatum attenuates appetitive behaviors, including self-administration of cocaine and other drugs of abuse. Yet it is unclear how the ventral striatum contributes to the selection or maintenance of behavior in either the normal or addicted state. In a new project, we plan to examine the relation between motivational state and neuronal signals in the ventral striatum and the network of structures connected to it, such as amygdala, hippocampus, etc.

Major findings:

To examine the motivational functions of the ventral striatum and associated structures, it is necessary first to differentiate neuronal signals related to the motivation to earn a reward from signals related to instrumental motor acts, appetitive behavior, and the reward process itself. We have devoted several months to the development of a behavioral paradigm that will permit such differentiation and also allow precise control of degree of motivation.

The task consists of a series of trials on which the animal is rewarded with juice after one, two, or three correct responses. On each trial, the monkeys are required to detect a change in the color of a small square (target stimulus) presented at the center of a video monitor and to release a manipulandum within 1 second after this color change occurs. A correct response is signaled by a second stimulus (reward cue), located above the target stimulus, and which remains on throughout the succeeding trial. An error results in the disappearance of both stimuli and a time-out period. The task differs from a variable-ratio paradigm in that the reward cue indicates the monkey's progress toward fulfilling the criterion for reward. The monkeys are trained initially on a schedule of continuous reinforcement, with the reward cue after a correct response being a constant bright white. Later, a variable-ratio schedule of reinforcement is introduced, with the brightness of the cue reflecting the proximity to reward. For example, if the upcoming criterion were three correct responses, the reward cue would start out at one-third the final brightness, increase to two-thirds after the first correct response, and then increase to full brightness after the second correct response. All three monkeys tested thus far have performed with the highest accuracy and shortest latency when the reward cue indicated that the next correct response

would be followed by juice. Since the perceptual demands during all trials are constant, the improvement in performance appears to reflect increasing motivation as the reward gets closer in time.

An unexpected finding is that the monkeys did not adopt a strategy that would maximize the number of rewards per trial. That is, the monkeys would have earned more rewards if they had ignored the reward cue entirely and treated each trial as though it would lead directly to reward. Instead, they performed with reduced accuracy and increased latency when this cue indicated that more work was still required. This suggests that the monkeys found it aversive to respond if there was no possibility of obtaining a reward on that trial.

When the reward given to one of the animals was changed from juice to intravenously administered cocaine, overall performance was markedly reduced both in error rate and reaction time. There are at least two possible interpretations of this result: Cocaine might be disruptive of performance of any task at any level of motivation, or it might interfere specifically with the mechanisms by which the costs and benefits of responding are weighed. We are currently performing control tests to decide between these alternative interpretations.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Disorders of motivation accompany many serious psychiatric and neurological disorders. Further, pharmacological agents that interfere with normal motivational processes have a devastating effect on behavior. This project focuses on the mechanisms that underlie motivation, with the initial goal of developing a quantitative measure that can be validated on both behavioral and neurophysiological grounds.

PROPOSED COURSE OF RESEARCH:

We will continue with both the behavioral analysis and single-neuron recording experiments in structures that have been implicated in motivation, such as the nucleus accumbens, and apply the techniques we have developed for analysis of multivariate data recorded from neurons in the visual system to these new multivariate data. The results will form the basis for simulations relating motivation to both neuronal function and other behavioral processes such as attention and memory, to guide the next stage of the research.

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

PROJECT NUMBER
Z01 MH 00471-37-LPP

PERIOD COVERED
October 1, 1991 to September 30, 1992

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

CO-PI: Loring J. Ingraham, Ph.D. Senior Staff Fellow LPP, NIMH

COOPERATING UNITS (if any)
Oranim Institute for Research on Kibbutz Education, Haifa University, Israel; Hebrew University Israel; University of Chicago, Illinois.

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL	OTHER
1.5	1.0	0.5

☒ (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-diciplinary 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 three 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.

Description

A. Other Personnel

Shaul C. Sohlberg, Ph.D.	Clinical Psychologist	Israel
Michael Natan, Ph.D.	Psychologist	Israel
Sol Kugelmass, Ph.D.	Oranim Institute Professor of Psychology, Hebrew University	Israel
Joseph Marcus, M.D.	Professor of Child Psychiatry, University of Chicago, Chicago, Illinois	
Bjorn Jacobsen, M.D.	Professor of Psychiatry ,Univ. of Copenhagen	

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 five years. Dr.Mirsky maintains contact with the Genains. Interest in the quadruplets continues to be high, as judged by the steady demand for photographs of them for psychology text books. Another article describing a new conceptualization of the Genains' life outcome has been published in *Progress in Experimental Personality Research.*

2. The Danish Adoptee Study

Previous annual reports have described our laboratory's studies of the adopted away children of schizophrenic parents raised by healthy adoptive parents, and studies of the biological extended families of adopted away schizophrenic individuals and controls (Z01 MH 02288-06 LPP).

During the past year, we have continued the analysis of current information about the adult outcomes of the adopted away children of schizophrenic parents, who have now passed through the major risk period for schizophrenia. These new data support our investigation of the longitudinal course of schizophrenic illness and the study of the role of environmental factors of the development of the development and course of schizophrenia.

3. The Israel Kibbutz--High Risk Study

This study, initiated in 1962, is a controlled longitudinal study of the development of psychopathology in the biological offspring of severely psychiatrically ill parents. Of 50 index children of an ill parent, half were raised conventionally with their parents in Israeli towns, and half raised by professional child care workers on kibbutzim. Matched control children were similarly divided between town and kibbutz. We have recently analyzed interviews with 85 of the

100 original subjects who have passed through the age of major risk for schizophrenia, and we have completed neuropsychological evaluation (including the LPP Attention Battery) of these subjects. We have also completed the contemporary assessment of a majority of the parents of our index and control probands.

We have prepared a speical issue of the *Schizophrenia Bulletin* detailing our findings.

Significance to Biomedical Research and to the Program of the Institute

The identification of the mechanism of the heritability of schizophrenia, and factors which modify its action, 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.

Another approach to identifying underlying mechanisms of schizophrenic illness lies in our work towards separating genetic forms of illness from non-genetic phenocopies. This allows for the development of more meaningful pedigree studies and the identification of relevant individuals for inclusion in studies seeking to identify molecular genetic variations in schizophrenia. These studies, 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: Dr. Mirsky maintains contact with the Genains and exchanges correspondence on a monthly basis. We have considered the possibility of inviting the Genains back to NIMH for another series of followup studies to include electrophysiological and other measurements that were not obtained during their visit in 1981.

Denmark: We plan to integrate diagnostic information from the contemporary assessment of adoptees' adult outcome with our base of longitudinal data on this population in order to study the role of environmental factors of the development and course of schizophrenia.

Israel: In the year ahead we plan to complete the editing and publication of a series of manuscripts in a special issue of the Schizophrenia Bulletin.

Publications

Mirsky AF, Ingraham LJ, Lowing PL (1992) Childhood stressors, parental expectation, and the development of schizophrenia. Prog Exp Perso Psychopathology Res 1992;15:110-130.

Ingraham LJ, Wender PH. Risk for affective disorder and alcohol and other drug abuse in the male and female biological relatives of affectively ill adoptees. J Affect Disord, in press.

Mirsky AF, Kugelmass, S, Ingraham, LJ . An overview of the NIMH twenty-five year longitudinal study of children at risk for schizophrenia. Schizophr Bull, in press.

Ingraham LJ, Kugelmass S, Frenkel E, Nathan M, Mirsky AF. Twenty-five year follow up of the Israeli high-risk study: current and lifetime psychopathology. Schizophr Bull, in press.

Ingraham LJ, Kugelmass S, Frenkel E, Natan M, Mirsky AF. Review and follow up of parental diagnoses in the Israeli high-risk study. Schizophr Bull, in press.

Ingraham LJ, Mirsky AF, Frenkel E, Natan M, Kugelmass S. Early predictorsof adult psychopathology in the Israeli high-risk study. Schizophr Bull, in press.

Mirsky AF, Ingraham, LJ, Kugelmass, S. Neuropsychological assessment of attention and its pathology in the Israeli cohort. Schizophr Bull, in press.

Frenkel E, Kugelmass S, Nathan M, Ingraham LJ. Locus of Control and resistance to psychopathology in children at risk for schizophrenia. Schizophr Bull, in press.

Nathan M, Kugelmass S, Frenkel E, Ingraham LJ. Psychometric measurement and prediction of psychopathology in the Israeli high-risk sample. Schizophr Bull, in press.

Kugelmass S, Frenkel E, Ingraham LJ, Mirsky AF. Psychophysiological measures in children at risk for schizophrenia. Schizophr Bull, in press.

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

PROJECT NUMBER
Z01 MH 00484-32 LPP

PERIOD COVERED
October 1, 1991 to September 30, 1992

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

Other: Allan F. Mirsky, Chief, LPP, NIMH
David Pickar, M.D., Chief, SCS, NSB, NIMH
Judith Rapoport, M.D., Chief, CHP, NIMH
Jordan Grafman, Ph.D., Chief, CN, MN, NINDS
C. T. Gordon, M.D., Clinical Associate, CHP, NIMH

COOPERATING UNITS (if any)
Child Psychiatry Branch, Experimental Therapeutics Branch, NIMH; Wards 3E and 4E

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
0.8	0.6	0.2

☒ (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 investigates the roles of autonomic nervous system (ANS activity, attention, and information processing and their interrelationships in the pathology, etiology, and prognosis of psychiatric disorders and studies their underlying biological and psychological processes. 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 various tasks, especially reaction time tests of attention. 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, MRI, and PET scans.

Recent and current work: 1) In schizophrenic patients we found that the atypical neuroleptic clozapine markedly attenuates ANS activity compared to a typical neuroleptic and placebo. Clozapine did not improve reaction time but there was evidence that it increased the efficiency of information processing, and this was mediated by a reduction of hallucinations. 2) Several methods to study respiratory sinus arrhythmia, an index of parasympathetic (vagal, cholinergic) control of heart rate have been developed and compared. Different methods are highly correlated, reliable, and resistant to body movements. 3) Parental 'expressed Emotion', an index of criticism and/or overinvolvement, is related, especially in fathers, to high skin conductance activity in obsessive-compulsive children. 4) Adolescents with Childhood Schizophrenia are being tested on the same protocol used with children with various other diagnoses. So far they show excessive spontaneous ANS activity but low Responsivity to novel and meaningful stimuli similar to our previous findings in adult schizophrenics. 5) Subjects with closed head injuries and with focal brain lesions are being tested on the same protocols used with the schizophrenics in part to explore the neurobiology of schizophrenia and in part to study central determinants of ANS reactions.

Project Description

A. Objectives

The major objective is further understanding of the role of autonomic nervous system (ANS) activity, information processing and attention, and their interrelationships in psychiatric disorders, primarily schizophrenia. The project involves studies of ANS and attention in relation to diagnosis and prognosis, studies of the effects of drugs and other therapeutic interventions, studies of the effects of various types of stress, and studies of the measurement of ANS activity.

B. 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 during rest, a series of nonsignal tones of constant or variable intensity, and performing tasks. Tasks include tests of attention using reaction time techniques, and tasks designed to be moderately stressful.

Adult and child schizophrenics are being tested on standard neuroleptics, placebo and clozapine. The effects of the alpha-2 antagonist idazoxin are now being studied. Reaction-time (RT) protocols include simple RT and a 'sensory dominance' paradigm. In this paradigm RTs are measured to lights and tones in simple and choice RT procedures, and on 'conflict' trials in which the light and tone are presented simultaneously. This paradigm tests the hypothesis that schizophrenics who have auditory hallucinations show an atypical bias to attend to auditory rather than visual stimuli.

As part of a larger LPP project, we are testing subjects with closed head injuries in the ANS and attention protocol used with schizophrenics. The purpose is to determine what specific brain areas may be involved in schizophrenic psychopathology. The ANS protocol is being used with subjects with focal brain lesions for the same purpose as in study. In addition, a series of pictures, some with strong emotional impact, are shown the subjects in order to confirm reports that patients with certain frontal lesions are autonomically unreactive to emotional stimuli.

A comparison of two methods of analyzing respiratory sinus arrythmia (RSA), purportedly an index of parasympathetic (vagal, cholinergic) activity is being carried out. A peak-to-trough measure of the heart rate changes occurring with each respiration cycle and a method based on spectral analysis of heart rate are compared on raw data and data that have been digitally filtered to remove low frequencies. We are attempting to study the effects of differences in motor activity by comparing analyses of just selected portions of recordings that are free of gross body movements with analyses of the total record. Analyses of the data from a study described in prior annual reports, on yohimbine, which purportedly increases parasympathetic activity, are planned. This should provide a good test of the validity of the various methods.

C. Major Findings

We have completed data analyses from 25 adult schizophrenic subjects, all of whom were tested on clozapine and either placebo or a standard neuroleptic. Twenty were tested on all three treatments. On the ANS measures, compared to both other treatments clozapine markedly reduced all aspects of electrodermal activity, increased heart rate, and decreased heart rate variability probably largely due to its anticholinergic and antihistaminic properties. However, vasomotor responses, which are thought not to involve cholinergic synapses, were similarly

attenuated, suggesting that clozapine may have central actions that reduce ANS activity. Attempts to predict which patients would improve on clozapine compared to the other two treatments were not markedly successful. The best predictor was that patients showing a good response to clozapine had a smaller increase in tonic ANS activity to a task when on the alternate treatments than did clinical nonresponders. Although clozapine attenuated this tonic ANS response compared to the other treatments, the good clinical responders to clozapine were less affected than the nonresponders. In general, the data suggest that a good clinical response to clozapine compared to a standard neuroleptic is accompanied by a selective focus of relatively greater ANS activity elicited by more important stimuli and/or situations and less by unimportant ones. We are continuing to collect data with this paradigm in order to attempt to replicate these results.

Eight of the 25 patients were too psychotic to perform the RT tasks on placebo. However, among the remaining patients, there was no evidence of improvement in RT by clozapine on the average in either paradigm compared to placebo, and RTs under the standard neuroleptic were nonsignificantly better than those on clozapine. On the sensory dominance paradigm patients, like normal controls, were vision-dominant as evidenced by faster RTs to lights than to tones on choice and conflict trials. These effects were significantly reduced under clozapine treatment. On conflict trials, subjects on placebo and neuroleptics showed more failures to respond within 2000 msec to the tone than to the light. This difference was significantly reduced on clozapine. In medicated patients non-responses to the tone were more frequent in those with hallucinations. The results suggest that clozapine may increase the ability of schizophrenics to process nondominant or unattended stimuli possibly by increasing the efficiency of resource allocation and that this may be partially mediated by a reduction in hallucinations. The data also suggest that subjects with hallucinations rather than being audition dominant as hypothesized may have a bias to disattend to external auditory stimuli. Two papers on the clozapine study are in preparation.

Preliminary results for the first 5 childhood onset schizophrenic patients tested on placebo show that, in comparison to a large group of age and sex matched controls, the patients showed excessive spontaneous autonomic activity but lower responsivity to novel and meaningful stimuli similar to our previous results with adult schizophrenics. They also show marked deficits in attention as indexed by reaction time tasks.

Using data from normal children and adolescents we have found that various measures of sinus arrythmia which have been proposed are highly correlated, highly reliable, and not greatly affected by body movements. We plan to use the technique on data from other patient studies.

Proposed Course

We are accumulating a large data-base in adult schizophrenia and are eager to determine the relationship of ANS variables to clinical and biological variables available on these patients. Many patients have PET scans near the time of our tests, and exploration of those relationships is a priority. There are several promising leads in the literature relating autonomic activity with type of symptoms, structural and functional brain abnormalities, biogenic amines, season of birth, clinical response to medication, etc. for which we have the data to test more definitively than perhaps can be done elsewhere.

Collection and analysis of data will continue for current projects on schizophrenic and nonschizophrenic psychopathology, in brain injured subjects, and in normal controls.

If reliable and valid measures of respiratory sinus arrythmia can be established, this will be applied to data from studies on Attention Deficit Disorder, Schizophrenia, and Affective Disorder, groups for which cholinergic deficits have been hypothesized.

Publications

Albus M, Zahn TP, Breier A. Anxiogenic properties of yohimbine: I. Behavioral, physiological, and biochemical measures. Eur Arch Psychiatry Clin Neurosci, in press.

Albus M, Zahn TP, Breier A. Anxiogenic properties of yohimbine: II. Influence of experimental set and setting. Eur Arch Psychiatry Clin Neurosci, in press.

Breier A, Albus M, Wolkowitz OM, Zahn TP, Paul S, Pickar D. The effects of psychological and physical stress in humans. In: Plotnikoff NP, Faith RE, Murgo AJ, Wybran J, eds. Stress and the Immune System. Caldwell, NJ: The Telford Press, 1991;47-60.

Gordon CT, Casanova M, Zamitkin A, Zahn T, Hong W, Rapoport JL. Childhood onset schizophrenia: Neurobiologic Characterization and Pharmacologic response; NIMH studies in progress. Schizophr Bull, in press.

Hibbs ED, Zahn TP, Hamburger SD, Kruesi MJP, Rapoport JI Parental expressed emotion and psychophysiological reactivity in disturbed and normal children. Br J Psychiatry 1992;160:504-10.

Zahn TP, Frith CD, Steinhauer SR. Autonomic functioning in schizophrenia: Electrodermal activity, heart rate, pupillography. In: Steinhauer SR, Gruzelier JH, Zubin J, eds. Handbook of Schizophrenia, Volume 5: Neuropsychology, Psychophysiology and Information Processing. Amsterdam: Elsevier Science Publishers, 1991;185-224.

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

PROJECT NUMBER
Z01 MH 00486-20 LPP

PERIOD COVERED
October 1, 1991 to September 30, 1992

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, NIMH

Other: Judith Rapoport, M.D., Chief, CHP, NIMH
Marcus Kruesi, M.D., Clinical Associate, CHP, NIMH

COOPERATING UNITS (If any)
Child Psychiatry Branch, NIMH, Ward 3E

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER
0.7	0.4	0.3

☒ (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.)

Autonomic nervous system (ANS) activity and attention has b een assessed in 34 boys with diagnoses in the Disruptive Behavior Disorder (DBD)spectrum (Conduct Disorder, Oppositional Defiant Disorder and/or Attention Deficit Hyperactivity Disorder [ADHD]), selected for their antisocial and disruptive behavior, and in 33 normal control boys. We recorded skin conductance (SC) and heart rate (HR) during a rest period, a series of nonsignal tones, and a simple reaction time (RT) task. Attention was assessed by two RT tasks which have been used extensively in schizophrenia research: 1) Simple RT with constant and variab le preparatory intervals, and 2) Simple, choice, and "crossmodal" RT to lights and tones.

The DBD boys had higher HR but no differences in SC indices of "arousal." However they had smaller increases in all baseline measures to the onset of the RT task than controls. SC response latencies were shorter in the DBD group despite slower RT. Their SC responses to signal stimuli habituated at a faster rate. These results are different from those obtained previously with more 'pure' ADHD samples. Correlational analyses with behavior ratings suggest that there may be autonomically hyperresponsive and hyporesponsive subgroups who may show clinical differences somewhat oblique to the Conduct Disorder subdiagnosis. In general, the data support the concept of the DBD spectrum. DBD subjects with low levels of metabolites of serotonin, norepinephrine, and dopamine measured from cerebrospinal fluid showed generally elevated ANS Responsivity, particularly in the RT task. The RT tasks showed that DBD boys have particular problems when the timing or modality of the stimulus is uncertain. A 2-year clinical followup of these subjects revealed that low SC level)age corrected) significantly predicted institutionalization. There were similar but nonsignificant trends for low ANS activity to predict poor outcome in this group for other variables.

The ANS and attention test are being repeated on a new sample of younger boys all of whom have a diagnosis of ADHS.

Project Description

A. Objectives

The project on DBD boys has two general objectives. One is to look for ANS and attention markers of diagnosis and subdiagnosis and to determine if the ANS data support the DBD spectrum concept. Previous research in this laboratory and others has shown that ADHD boys do not differ from normals in baseline indices of arousal but have generally lower ANS responsivity to stimuli. The literature on psychopathic personality and criminality in adolescents and adults -- outcomes for which boys in the DBD spectrum, particularly those with Conduct Disorder (CD), are at risk -- shows evidence of low ANS base levels and diminished SC (but not HR) reactivity to stimuli, particularly aversive stimuli. Our ANS protocol allows tests of some of these differences in the boys. A second objective is to study individual differences among the DBD boys with an eye to develop predictors of outcome. We also have tried to explicate the attention deficit in ADHD children.

B. Methods Employed

The general methods of these studies include measurement of ANS activity through skin conductance (SC) and heart rate (HR) during rest, a series of nonsignal tones of constant or variable intensity, and performing tasks. Tasks include tests of attention using reaction time techniques. The more complex versions of these are given without physiological recording. Subjects are given a thorough clinical workup by the Child Psychiatry Branch, including ratings of aggression and impulsivity by teachers, parents, doctors, and the child him/herself.

C. Major Findings

We have completed testing and most data analysis on 34 DBD and 33 control boys. The ANS results, described in more detail in last year's annual report, show that there was no overall difference in ANS 'arousal', but that the DBD and control boys showed significantly different profiles of three conventional indices of ANS activity: DBD boys were significantly higher in HR, nonsignificantly higher in spontaneous SC responses, but nonsignificantly lower in SC levels. The DBD boys did show significantly attenuated tonic increases in all three arousal measures at the onset of a simple reaction-time (RT) task. Their SC responses to the RT signal stimuli relative to those to the RT ready signal were larger than those of controls on the first block of trials, but they habituated markedly faster than the controls in terms of both frequency and amplitude over the 18-trial series. Paradoxically, the latencies of these SC responses were significantly faster in the DBD group than in controls despite slower RT. These data provide some evidence of faster development of 'cortical inhibition' in these subjects as predicted by some theories. However, a lack of behavioral inhibition is indicated by many more extraneous button presses between trials during the RT task by the DBD boys.

Individual differences within the DBD group were investigated by comparing boys with and without a diagnosis of Conduct Disorder (CD) and by correlations with various measures of aggression and impulsivity. As expected, CD boys had lower ANS baseline values than non-CD subjects. A problem with the ratings was that those made by different raters did not correlate with each other. Aggression rated by or from information from parents and the child himself did not correlate with baseline ANS 'arousal' measures, but aggressive boys by those ratings showed generally diminished HR responsivity in all phases of the study. Aggression ratings from the boys' home teachers correlated negatively with ANS arousal, while those from the NIH ward teacher correlated positively with arousal. Thus either the different raters (and rating scales) are measuring different constructs or ANS activity may be partially related to the

context in which aggressive behavior occurs. Although there were also some inconsistencies in impulsivity ratings several measures were positively correlated and none were negatively correlated with ANS arousal. The general conclusions from this study are first, that the DBD spectrum concept hangs together rather well since there are few differences between CD and non-CD boys (except on some baseline variables) even on variables that distinguish the DBD group from controls, and second, that individual differences within the DBD spectrum might be more fruitfully described by behavior dimensions rather than by conventional subdiagnoses.

We reported a number of correlations between monoamine metabolites from the CSF and ANS variables in last year's annual report. In general, they showed that high values of the metabolites were significantly related to low ANS responsivity, but not to baseline values. Very preliminary results from a new sample of ADHD boys (N=17) are generally confirmatory, but, by themselves they are not significant.

The results of the extended RT protocols were detailed previously and have been published.

Dr. Kruesi of CHP has completed a clinical 2-year followup on 29 of the above subjects, and we have examined four ANS variables recorded at time 1 as predictors of various outcome variables. Generally, low baseline ANS activity was related to poor outcome, but the only significant relationship after age correction was between low SC levels and institutionalization. Low spontaneous SC activity and HR also predicted institutionalization, but marginally. Low HR was marginally related to suicide attempts. However slow habituation of the SC orienting response was related marginally to physical aggression and poor adjustment at followup. The data provide some confirmation of previous findings in the literature of associations between low ANS activity in children and adolescents and future suicidal and antisocial behavior.

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

The ANS data on the DBD group are different from those in previous studies in this laboratory and elsewhere on samples of less disruptive ADHD children, suggesting biological differences may exist within the DBD spectrum. The data from the followup study, in conjunction with those from the group as a whole in comparison with normal subjects, are also suggestive of heterogeneity within this group. This study should help us understand how cognitive, biological, and personality factors are interrelated in these children.

This study may also help determine the mechanisms of ANS measures through the correlations with the neurobiological data. This study is relevant to a major objective of LPP to develop a taxonomy of attention disorders.

E. Proposed Course

A paper on the comparison of the ANS data for DBD and normal boys is under review. We are planning to use those data to compare with similar data for obsessive-compulsive (OCD) children and adolescents. We plan to delay publishing the monoamine data until we complete analyses of similar OCD data.

A new sample of younger ADHD children is being tested on similar protocols.

Publications

Kruesi MJP, Hibbs ED, Zahn TP, Keysor CS, Hamburger SD, Bartko JJ, Rapoport JI. A two year prospective follow-up study of children and adolescents with disruptive behavior disorders: Prediction by CSF 5-HIAA, HVA, and autonomic measures? Arch Gen Psychiatry 1992;49: 429-35.

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

PROJECT NUMBER
Z01 MH 00491-16 LPP

PERIOD COVERED
October 1, 1991 to September 30, 1992

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., Ph.D., Guest Researcher, LPP, NIMH
Judith Rapoport, M.D., Chief, CHP, NIMH
Markus Kruesi, M.D., Clinical Associate, CHP. 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 STAFF YEARS	PROFESSIONAL	OTHER
0.1	0.1	0.0

☒ (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, reaction time, and autonomic nervous system (ANS) activity in normal humans. Bilateral skin conductance and heart rate have been recorded in sessions in which constant and variable intensity tones and lights are presented, in which auditory and two-flash thresholds (TFT) determined by methods which permit signal detection analyses and in which simple reaction time is measured. Several standardized personality tests were also given. These include scales of sensation-seeking, the Eysenck scales of extraversion, neuroticism, and psychoticism, field dependence and anxiety. In addition comprehensive measures of lateral dominance have been given. A procedure for manipulating ANS arousal experimentally with minimal distracting effects -- a change in posture from supine to standing -- was 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.

Recently, personality variables assessed chiefly from the Eysenck scales, including scales of impulsivity, empathy, and venturesomeness have been correlated with ANS activity in normal children and adolescents. The most consistent results are negative correlations between Extraversion and both phasic and tonic electrodermal responsivity to both nonsignal and signal stimuli and to the onset of a task period.

Project Description

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").

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, reaction time, and ANS activity in normal humans. Other objectives are 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 of increasing arousal by means of a postural change.

Methods Employed

Over 200 adult 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. More recently a protocol similar to that used with patients in Z01 MH 00484 LPP has been used in which ANS recording is done during a rest period, a series of innocuous tones, and a fixed foreperiod reaction time task. Using similar protocols should facilitate comparison with patient data.

Major Findings

A study in which the Eysenck scales mentioned above and scales of impulsivity, empathy, and venturesomeness were correlated with ANS activity in 32 normal children and adolescents (20 males and 12 females) was described in some detail in last year's annual report. This showed rather consistent and significant correlations between low ANS responsivity to a variety of stimuli and the Eysenck dimension of extraversion. Such relationships did not occur in a sample of boys with Disruptive Behavior Disorders, but since the normal sample included girls, and girls were strong contributors to the relationships observed, the meaning of the differences between groups is not clear. Before deciding how to publish these findings we are planning to look at the same relationships in a group of obsessive-compulsive children and adolescents which includes subjects of both sexes.

Significance to Biomedical Research and the Program of the Institute

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 reduce the large number of variables generated by our analysis programs. To this end we plan to apply multivariate analyses such as multiple regression and/or confirmatory factor analysis to the large data set on normal adults.

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.

Publications

None.

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

PROJECT NUMBER
Z01 MH 00503-12 LPP

PERIOD COVERED
October 1, 1991 to September 30, 1992

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, Ph.D. Chief, LPP, NIMH

Co. PI: Connie C. Duncan, Ph.D. Research Specialist

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
1.5	.75	.75

☒ (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 Description

Brainstem Mechanisms in Attention Impairment

We have published work indicating that there are disturbances (prolonged transmission time) in the processing of auditory information in the brain stem in some subjects with infantile autism and in schizophrenia. We have also shown that in absence seizures (characterized by spike-wave activity in the EEG), both naturally-occurring and experimentally-induced, there may be perturbations of auditory brainstem functioning.

We have recently completed the reanalysis of brain stem auditory evoked potentials (BAERs), recorded in the interictal period, from a group of eight patients with absence epilepsy. These subjects were matched in terms of age, sex and educational level with a group of normal controls. The data indicate that, as in the case of the ictal period in such cases, there are significant prologations of several of the BAER components in the patients.

We have also recently completed an analysis of BAER data from schizophrenic subjects. We have found significant latency increases in waves III, IV and V in the patient group. Moreover, an additional analysis has revealed a tendency for longer latencies to be present in medicated than unmedicated subjects.

These findings provide evidence for altered auditory brain stem functioning in schizophrenic patients.

We are also gathering BAERs in patients with head injuries; none of these data have as yet been analyzed.

Neurobehavioral Studies in Absence Epilepsy

A group of 10 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. The 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. Some of these data have been published by Duncan, or as part of the proceedings of a symposium on generalized epilepsy. We are continuing this work at present by testing a control group of patients with complex partial seizures.

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 elsewhere.

We have been able to administer attention tests to a group of children with absence epilepsy and to their siblings and parents. The results formed the Ph.D. dissertation of Dr. Miriam Levav, who found expected attentional deficit in the probands and a somewhat unanticipated result of a similar deficit in the mothers (but not the fathers or the siblings) of the probands. The results have some bearing on the nature of the genetic transmission of the disorder.

A Taxonomy of Attentional Disorders

During the past three years, 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 203 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 an 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.

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.

Publications

Freedman R, Mirsky AF. Evoked potentials: exogenous components. In: Zubin J, Steinhauer SR, Gruzelier JH, eds. Handbook of schizophrenia, vol. 4. Experimental psychopathology, neuropsychology and psychophysiology. Amsterdam: Elsevier, 1991;71-90.

Mirsky AF, Duncan CC. Attention impairment in human clinical disorders: schizophrenia and petit mal epilepsy. In: Sheer DE, Pribram KH, eds. Attention: theory, brain functions and clinical applications. Hillsdale, NJ, Erlbaum, 1992, in press.

Mirsky AF, & Bakay Pragay E. Brain stem mechanisms in the processing of sensory information: clinical symptoms, animal models and unit analysis. In: Sheer, DE & Pribram, KH, eds. Attention: Theory, brain functions and clinical applications. Hillsdale, N.J.: Erlbaum, 1992, in press.

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

PROJECT NUMBER
Z01 MH 00508-10 LPP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Neuropsychological Evaluation of Psychiatric and Neurologic Patients

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
Co-PI:	Barbara P. Jones, Ph.D.	Neuropsychologist, LPP, NIMH
Co-PI:	Connie C. Duncan, Ph.D.	Research Specialist, LPP, NIMH

COOPERATING UNITS (if any) BPB, NIMH; MNB; MN; NINDS; LN; NIMH; Chestnut Lodge Hospital; Johns Hopkins University; Maryland Head Injury Foundation; Medical College of Virginia; Virginia Commonwealth University; University of Washington; University of Pittsburgh

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
2.5	1.0	1.5

☒ (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, and children ages 6-16. The data provided by these batteries are being used to construct a neuropsychological theory of the elements of attention that may be applied to the neurological and psychiatric diagnostic groups under study in the LPP. The LPP has as its major focus disorders involving impaired attention, including schizophrenia, epilepsy, eating disorders, affective disorders, head injuries, and AIDS dementia complex. 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 electrophysiological, neuroradiological, and biochemical information.

Major Findings

A test battery or parts thereof, to assess neuropsychological functions has been administered to over 500 subjects in our laboratory. Outside the laboratory, over1000 tests have been given in a collaborative study with the Johns Hopkins School of Hygiene and Public Health.

A factor analysis-derived model of the elements of attention, based on neuropsychological tests, has been developed. It has proven very useful in the interpretation and the design of research in our laboratory and in studies being conducted with collaborators outside of NIMH. The essential factor-analytic results that led to this model have been replicated by four other teams. In addition to the research mentioned in this report, outside collaborations include study of the long-term behavioral effects of maternal alcohol consumption in Seattle, long-term consequences of exposure to lead in Boston and Pittsburgh, and neurocysticercosis in Ecuador. A comprehensive review and description of the attentional elements has been published in *Neuropsychology Review.* The results of these other collaborations will be discussed in future annual reports.

Studies in Adults: The Genetic Epidemiology of Schizophrenia in Ireland

This is an ideal location for such a study because of the excellent health registers, the cooperative nature of patients and health professionals, the fact that English is the native language and the stability of the population.

We have completed analysis of test scores on our "attention battery" on a group of 26 schizophrenics (S), 39 of their first-degree relatives (SR), and 46 matched controls (C), in Roscommon, Ireland. The total N is 111. The groups are closely matched in mean age and educational level. The S group is clearly impaired on most tests. On most tests, the SR group's scores fall between those of the S and C groups, suggesting that the relatives share, albeit in milder form, the attentional impairment of the schizophrenic probands. Certain tests, however, appear to be particularly discriminating in terms of statistical separation among the S, SR and C groups.

A preliminary report on these and related data is in press in the *Journal of Psychiatric Research.*

Panic Disorders

The LPP is continuing a study of neuropsychological functioning in panic disorder. A number of studies have demonstrated biological differences between patients with panic disorder and normal controls; concordance rates in twins support a genetic origin for panic disorder, and the transmission pattern is consistent with a single-locus model. A recent neuropsychological study of patients with panic disorder found impairments in visual learning and visual recall, consistent with abnormality in the right parahippocampal region, but did not find deficits in verbal learning or attentional capacity.

We hope to test a total of 12 to 20 patients with panic disorder and an equal number of normal control subjects.

Studies in Children: Childhood Attentional Disorders-The Prevention Research Center

The goal of this project is to understand attention in young children, its developmental course, its interaction with current adaptive behavior, and its relationship to future maladaptive behavior and psychopathology. We have now completed the third assessment of attention functions in a group of 300 children from East Baltimore in conjunction with the Prevention Research Center (PRC) of Johns Hopkins University.

Analysis of the data from the first two assessments suggests relationships among teacher-rated aggressive, shy and concentration behaviors and the "elements" of attention derived from our test battery.

Information from these studies was used to develop an assessment and preventive intervention program aimed at improving both attentive competence and classroom concentration behavior in the first grade. This proposal formed a key part of the renewal application of the PRC which was recently funded by NIMH for another five years.

Genetic/Famial Influences in Absence (petit mal) Epilepsy

We have recently completed a study of attentional performance in patients with absence epilepsy and their first-degree relatives (siblings, parents). The data suggest greater attention impairment in female than male probands, and a similar pattern in their unaffected parents (i.e., mothers score more poorly than fathers). We are seeking additional families with an epileptic proband to test the specificity of this finding.

Proposed Course

In the coming year we are planning to increase our sample sizes for several of the ongoing studies, in order to provide greater power to our analyses.

Specific new collaborations that are being implemented include one with the Department of Psychiatry-Division of Epidemiology of Johns Hopkins University (Dr. Ann Pulver). This investigator has a cohort of 500 schizophrenics and 1000 first degree relatives that we are planning to evaluate with the LPP attention Battery.

In addition, we are engaged in an attention assessment program for children experiencing academic and conduct difficulties who are referred by the teachers in the D.C. school system. This project will be conducted in collaboration with Dr. Trudy Summers and other staff psychologists at a D.C. Mental Health Services facility in Northwest Washington.

Publications

Freedman R, Mirsky AF. Evoked potentials: exogenous components. In: Zubin J, Steinhauer SR, Gruzelier JH, eds. Handbook of schizophrenia, Vol. 5, Experimental psychopathology, neuropsychology and psychophysiology. Amsterdam: Elsevier, 1991;71-90.

Jones BP. Repression: The evolution of a psychoanalytic concept from the 1890s to the 1990s. J Am Psychoanal Assoc, in press.

Jones BP, Butters N. Neuropsychological assessment. In: Hersen M, Kazdin AE, Bellack AS, eds. The clinical psychology handbook. 2nd ed. New York: Pergamon, 1991, 406-429.

Jones, BP, Duncan, CC, Brouwers, P & Mirsky, AF. Cognition in eating disorders. J Clin Exp Neuropsychol 1991;13, No. 5:711-728.

Mirsky, AF & Bakay Pragay, E. Brain stem mechanisms in the processing of sensory information: Clinical symptoms, animal models and unit analysis. In Sheer DE, & Pribram KH eds. Attention: Theory, Brain Functions and Clinical Applications. Hillsdale, N.J.: Erlbaum, 1992, in press.

Mirsky, AF & Duncan, CC. Attention impairment in human clinical disorders: Schizophrenia and petit mal epilepsy. In Sheer DE, & Pribram KH eds. Attention: Theory, Brain Functions and Clinical Applications. Hillsdale, N.J.: Erlbaum, 1992, in press.

Mirsky, AF & Siegel, A. The neurobiology of violence and aggression. Publications of the National Research Council., 1992, in press.

Mirsky, AF, Anthony, BJ, Duncan, CC, Ahearn, MB, & Kellam, SG. Analysis of the elements of attention: A neuropsychological approach. Neuropsychol Rev 1991;2:109-145.

Mirsky, AF, Lochhead, SJ, Jones, BP, Kugelmass, S, Walsh, D, & Kendler, KS. On familial factors in the attentional deficit in schizophrenia-a review and report of two new subject samples, J Psychiatric Res, 1992, in press.

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

PROJECT NUMBER
Z01 MH 00509-10 LPP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Attention Disorders as Assessed by Brain Event-Related Potentials

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

Co. PI: Connie C. Duncan, Ph.D., Research Specialist, LPP, NIMH
Others: Mary Kosmidis, Ph.D., Staff Fellow, LPP, NIMH
Bruno Anthony, Ph.D., Guest Researcher, LPP, NIMH

COOPERATING UNITS (if any) Clinical Psychobiology Branch, Neuropsychiatry Branch, Child Psychiatry Branch, Laboratory of Clinical Science, NIMH; Medical Neurology Branch, NINCDS; Chestnut Lodge Hospital; University of Illinois; University of Maryland

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL	OTHER
3.1	1.1	2.0

☒ (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 brain event-related 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 brain event-related potentials and performance on cognitive tasks are used to differentiate patterns of dysfunction in attentive mechanisms in subjects with diagnoses of schizophrenia, seizures, seasonal affective disorder, eating disorders, dyslexia, closed head injury, attention deficit hyperactivity disorder, and Tourette's disorder. A related objective of this project is to differentiate state versus trait attributes of these disorders to increase understanding of their etiologies. Brain event-related 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. Correlations between brain event-related potentials and neuroimaging (magnetic resonance imaging) data are used to study the relationship between indices of information processing and the volume of brain regions implicated in the pathophysiology of schizophrenia. Psychological correlates are investigated by relating the data to extensive neuropsychological, psychiatric, and personality measures as well as to performance on behavioral tasks.

Major Findings

Schizophrenia

To date, we have tested over 120 patients (including approximately 50 unmedicated) who met *DSM-III* criteria for schizophrenic disorder. The data show that, as in previous studies, the P300 was smaller in the schizophrenic patients than the normal controls. Moreover, this difference was most striking for stimuli in the auditory as compared with 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 schizophrenics. Change in P300 amplitude in the visual modality was significantly correlated with favorable medication response. There was no correlation between auditory P300 amplitude and clinical response. The data thus suggest that reduced visual P300 amplitude may be a *state* marker whereas reduced auditory P300 may qualify as a *trait* marker of schizophrenia.

In collaboration with Drs. Suddath and Egan, the size of brain structures as measured by MRI was examined in relation to ERP indices of information processing in a group of 16 medicated schizophrenic patients. Analysis of the data indicates that the highest correlations are found between the amplitude of the visual P300 and the volume of the right hippocampus (r = .50 to .75, $p < .05$ to .005 across recording sites). In contrast, the amplitude of auditory P300 is correlated with the volume of the third ventricle (r = -.50 to -.60, $p < .05$). These findings suggest that indices of information processing are associated with the volume of brain regions (i.e., limbic structures) implicated in the pathophysiology of schizophrenia.

Absence and Complex Partial Epilepsy

Brain ERPs were used to study information processing in the interictal period in a group of patients with absence epilepsy, another group of patients with complex partial epilepsy and a matched group of normal controls. ERPs were recorded during performance of visual and auditory versions of the continuous performance test (CPT) of sustained attention.

Absence patients are greatly impaired in their ability to sustain attention. The behavioral differences seen on the CPT were paralleled in the P300 amplitude data, suggesting that the failure of absence patients to perform efficiently on the CPT is due, at least in part, to inability to mobilize and sustain attentional capacity. This was most pronounced in the auditory versions of the task. In most respects the patients with complex partial seizures resembled the controls, or had scores midway between the controls and the absence patients.

Seasonal Affective Disorder

Seasonal affective disorder (SAD) is a syndrome characterized by recurrent major depressions during the fall and winter months. The winter depressive symptoms can be treated by exposure of the eyes to bright artificial light. This sensitivity to light suggests the importance of studying visual information processing in these patients. We used the P300 component of the ERP to investigate whether clinical improvement following light therapy is associated with increased visual attention. Auditory ERPs were also recorded to assess the specificity of changes in attention.

Depression in the patients decreased significantly following light therapy ($p<.0001$). In the visual modality, SAD patients who exhibited the most clinical improvement following light therapy showed the greatest increase in P300 amplitude ($r=-.71$, $p<.005$). In contrast, clinical response was uncorrelated with changes in P300 to auditory stimuli ($r=-.18$). No changes were seen in the normal controls. The results indicate that a positive clinical response to light therapy in SAD patients is associated with a significant increase in the attentional resources that are mobilized to process visually-guided information.

Because the change in visual P300 amplitude occurs rapidly (within a couple of days of the initiation of treatment), it may be an effective predictor of those who will benefit from this

therapy. Moreover, P300 may aid in establishing subcategories of affective disorders that are responsive to phototherapy.

Seasonal Effects on P300

Deldin, Duncan, and Miller (1989) reported that the amplitude of P300 varies as a function of gender and season of testing. Specifically, they found that P300 is larger in women than in men and larger in summer and winter than in spring. We sought to replicate their findings using a within-subject design, in which P300 was assessed in the same subjects in different seasons.

Results on our preliminary sample of 10 subjects failed to replicate the specific findings of Deldin et al. (1989). Namely, there were no effects of season or gender and no interaction of season and gender on P300. However, our subjects were not tested in the spring, when amplitudes had been observed to be minimal. In a more general sense, our data do support their results, in that the scalp distributions of both visual and auditory P300 were found to vary as a function of season.

Eating Disorders

We used ERPs to assess aspects of information processing in patients with eating disorders: anorexia nervosa or normal weight bulimia. A group of matched normal controls was also studied.

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 when task demands were maximal. Bulimics were not distinguishable from normal women on any of the measures. Preliminary findings after long-term weight restoration in a separate group of 15 anorexic patients indicate reversal in some of the ERP abnormalities.

Dyslexia

Reduced amplitude P300 has been observed in children with reading disorders. Our study was aimed at assessing whether the P300 reduction observed in dyslexic children would also be found in dyslexic adults. Moreover, we used auditory and visual stimuli, presented in separate reaction time tasks of graded difficulty, to evaluate whether any differences were independent of modality and attentional demands.

No group differences in P300 were seen under relatively undemanding task conditions. However, the dyslexic group showed a widespread reduction in visual P300 amplitude in the choice reaction time tasks.

Additional analyses revealed that dyslexics with a probable history of attention deficit disorder (ADD) accounted for the group differences in P300; the dyslexics without a history of ADD were indistinguishable from controls at all electrode sites. Furthermore, whereas the non-ADD dyslexic subjects showed the same pattern of hemispheric involvement of P300 as controls in both modalities (right>left), a reversed asymmetry in auditory P300 was apparent for the ADD dyslexic group. The results thus suggest that a distinct brain organization may characterize those dyslexic adults who have a history of concomitant attention deficit.

Attention Deficit Hyperactivity Disorder and Tourette's Disorder (TD)

We have completed data collection in a study comparing unmedicated, 7-to 11-year-old children with a diagnosis of (a) ADHD, (b) TD, or (c) both ADHD and TD. In addition to the clinical groups, 15 control children were studied.

The amplitude of the P300 in normal control children was significantly greater than that in children with ADHD. However, neither the TD nor the TD-ADHD group differed from controls. This finding suggests that although children with TD often possess attentive problems consistent with a diagnosis of ADHD, these difficulties seem to result from different information processing difficulties than those seen in children with ADHD.

Significance to Biomedical Research and the Program of the Institute

Since attentional deficit and cognitive dysfunction are characteristic of many

neuropsychiatric disorders, it is important to develop a precise empirical and theoretical account of these symptoms. 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.

Proposed Course

We are currently completing data collection on our studies of patients with schizophrenia and closed head injury; the within-subject investigation of seasonal effects of P300 is ongoing. Data collection is complete and analysis is in progress on our studies of eating disorders, epilepsy, dyslexia, ADHD and TD. Our studies of seasonal affective disorder on P300 have been completed.

We plan to expand our investigation of the interrelation among ERP components and neuropsychological and MRI variables. We plan also to begin testing first-degree relatives of psychiatric patients to determine whether the ERP is a marker of specific disorder. We have implemented selected studies with head-injured cases to test hypotheses derived from various clinical groups concerning the involvement of brain structures in the pathophysiology of psychiatric disorders. Electrophysiological predictors of clinical response to psychopharmacological and other forms of treatment will be sought, as patient availability allows.

Publications

Gaist PA, Oberzanek E, Skwerer RG, Duncan CC, Shultz PM, Rosenthal NE. Effects of bright light on resting metabolic rate in subjects with seasonal affective disorder and control subjects. Biol Psychiatry 1990; 28:989-996.

Duncan CC, Mirsky AF, Deldin PJ, Skwerer RG, Jacobsen FM, Rosenthal NE. P300 as an index of treatment response in seasonal affective disorder. In: Stefanis CN, Soidatos CR, Rabavilas AD, eds. Psychiatry: A world perspective (Vol. 2). Amsterdam: Elsevier Science Publishers B.V., 1990; 398-401.

Mirsky AF, Anthony BJ, Duncan CC, Ahearn MB, Kellam SF. Analysis of the elements of attention: A Neuropsychological approach. Neuropsychol Rev 1991; 2:109-145.

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Duncan CC. Basic cognitive and clinical studies of P300. In: Kaga K, Hiramatsu K, Osawa M, Koga Y, eds. A manual for basic and clinical P300 research. Tokyo: Shinohara Publishing Company, in press.

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

PROJECT NUMBER
Z01 MH 02288-08 LPP

PERIOD COVERED
October 1, 1991 to September 30, 1992

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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CO-PI: Loring Ingraham, Ph.D, Senior Staff Fellow, LPP, NIMH
Others: Paul Wender, M.D., Professor of Psychiatry, University of Utah
Bjorn Jacobsen, M.D., Professor of Psychiatry, University of Copenhagen
Dennis Kinney, Ph.D., Assistant Professor of Psychiatry, Harvard University

COOPERATING UNITS (If any)
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TOTAL STAFF YEARS.	PROFESSIONAL	OTHER
1.8	1.5	0.3

☒ (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 nature and strength of genetic and environmental factors in etiology. Where these factors are separated by the process of adoption, specific etiologic hypotheses can be tested separately and in combination. As reported in previous annual reports, classical schizophrenia and schizophrenia-like disorders occur at a significantly elevated rate in the biological relatives of chronic schizophrenic adoptees and not in their adoptive relatives, a finding now replicated in our national register of the nearly 15,000 Danish adoptees who have reached maturity. This provides compelling evidence for the significant operation of genetic factors in the etiology of this disorder.

Our demonstration that the increased prevalence of schizophrenia in the biological families of adoptees does not differ significantly from that found in the natural families of schziophrenics indicates that the well established familial tendency in this disorder is an expression of genetic factors, and provides hitherto lacking justification for the use of family studies to examine the modes of genetic transmission and to search for genetic linkages in large pedigrees of naturally reared schizophrenics.

Adoption Studies Assessing Etiological Factors in Mental Illness

Studies Demonstrating Heritable Risk for Schizophrenia

In work initiated by Drs. Kety, Rosenthal and Wender in the intramural program of the NIMH, investigation of the prevalence of schizophrenic illness among the biological relatives of schizophrenic adoptees in Copenhagen and the remainder of Denmark clearly implicated the operation of heritable factors in the liability for schizophrenia and schizophrenia spectrum illness. The prevalence of a disorder in the biological relatives of adoptees with that disorder in comparison with biological relatives of control adoptees offers a useful test for the expression of genetic factors in the disorder, but also a much needed evaluation of the validity of diagnoses based on clinical observation. The most recent findings from this project are described in a manuscript in press, and will be further elaborated in future manuscripts from this laboratory.

A Common Heritable Risk for Affective Disorder and Substance Abuse

Dr. Ingraham, in collaboration with Dr. P. Wender, this year investigated the risk for substance abuse in the biological relatives of adoptees with affective illness, controlling for potential confounds, and additionally assessed risk by probands' and relatives' gender. Both affective illness and substance abuse were more common in the biological relatives of affectively ill adoptees than in controls' relatives. Affective illness was more common than substance abuse among female index biological relatives, with the opposite pattern observed among male relatives.

Behavior Genetics of Affect

In work initiated and carried out by by Dr. Ingraham, the genetics of specific behaviors associated with psychiatric diagnoses in both normal and ill populations are being investigated. This approach, which tightens the focus of our investigations from a molar diagnosis-wide approach to a focus on specific behaviors has the potential elucidate the operation of genetic factors in psychiatric illness. Initial results of this work suggest the operation of genetic factors in shyness.

Specifying the Boundaries of the Schizophrenia Spectrum

The limited success of recent applications of molecular genetic techniques to schizophrenia may to a considerable extent be attributed to the looseness of the diagnostic categories that have been employed and the genetic heterogeneity thus displayed. Dr. Ingraham is analyzing the interview data in the adoption studies to specify more accurately the symptoms and manifestations found among the classical schizophrenic probands, with the aim of increasing the sensitivity and specificity of the diagnosis of schizophrenia. This same analysis applied to the biological relatives of the probands will be used to identify traits genetically associated with schizophrenia and to develop specific characterization of the syndrome. Dr. Ingraham continues to focus closely on the utility and validity of separating familial from non-familial cases of schizophrenia in order to specify more accurately potential variants of this illness.

Cerebral morphological and functional imaging in schizophrenia

Functional imaging of the brain has recently become possible through visualization and quantification of regional cerebral blood flow and metabolism using principles developed by Drs. Kety and Sokoloff or the IRP. Two manuscripts describing the fidelity and basis of the coupling between these functions and the quality of physiological or mental activity were published this year.

Significance to Biomedical Research and to the Program of the Institute

Our findings of a strong and quite specific genetic influence in the transmission of classical schizophrenia 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, and support continuing molecular approaches to gene linkage and location.

Our ongoing investigation of the extent to which specific syndromes and behaviors may be under genetic control strengthens links between the molecular and behavioral levels of analysis in psychiatric research.

Proposed Course

A third sample of schizophrenic adoptees has been identified in Denmark, representing adoptees with onset of illness and hospitalization subsequent to the previous search through the adoption and psychiatric registers. A cohort of matched controls is being selected. The biological and adoptive relatives of this sample are being examined for mental illness.

Fine grained analyses at the level of behavior, affect, and symptoms we are now conducting will build on extant analyses to investigate heritable aspects of psychiatric illness. We hope to use the results of these studies to guide the specification of affected individuals in the pedigrees of schizophrenic probands to focus future molecular genetic analyses.

Arrangements have been made for obtaining immortalized cell lines from members of selected pedigrees in the Danish adoption samples for linkage studies by members of the Section on Molecular Neurogenetics of the Clinical Neuroscience Branch.

Publications

Kety SS. The early history of the coupling between cerebral blood flow, metabolism, and function. In: Lassen NA, Ingvar DH, Raichle ME, & Friberg L. eds. Brain Work and Mental Activity, Copenhagen: Munksgaard, 1991;19-29.

Kety SS. The circulation, metabolic and functional activity of the human brain. Neurochem Res 1991; 6:1073-1078.

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Ingraham LJ, Wender PH. Risk for affective disorder and alcohol and other drug abuse in the male and female biological relatives of affectively ill adoptees. J Affect Disord, in press.

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

PROJECT NUMBER
Z01 MH 02295-07 LPP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 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: Allan F. Mirsky, Ph.D., Chief, LPP, NIMH
Co-PI: Frances H. Gabbay, Ph.D., Guest Researcher, USUHS
Others: Connie C. Duncan, Ph.D., Research Specialist, LPP, NIMH
Chris-Ellyn Johnson, Ph.D., Addiction Research Center, NIDA

COOPERATING UNITS (if any)
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Addiction Research Center, National Institute on Drug Abuse

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
0.2	0.1	0.1

☒ (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 these studies is to examine factors which affect vulnerability to alcohol and other substance abuse disorders. The aims of the family history study are (1) to compare the response to an acute alcohol challenge in individuals at low and high risk for substance abuse as defined by family history of alcoholism, (2) to compare the response to a stimulant drug (*d*-amphetamine) in the same groups, and (3) to examine the concordance of response to a stimulant and a sedative drug within subjects. Response to drug will be measured using a battery of measures including resting EEG, event-related potentials, the Continuous Performance Task, physiological tremor and standing stability, and self-reported subjective effects.

The aims of the drug preference study are (1) to test the hypothesis that individuals who prefer *d*-amphetamine over placebo will report more stimulant like subjective effects, and (2) to test the hypothesis that individuals who prefer *d*-amphetamine over placebo will show behavioral and electrophysiological responses consistent with the stimulant-like subjective effects, while individuals who prefer placebo will have a different profile of behavioral and physiological effects. Response to drug will be measured using the same battery of measures as will be used in the family history study.

The twin study of sustained attention will (1) permit estimates of the heritability of sustained attention, (2) examine developmental changes in that heritability, and (3) disentangle the effects of heredity and environment on the observed gender differences in the Continuous Performance Test. Knowledge of the genetic effects on the CPT will help to clarify the role of attention deficits in substance abuse and other disorders.

A. Overall Objectives

These studies are being conducted in collaboration with the Uniformed Services University of the Health Sciences and are funded in part by grants to the Department of Medical Psychology/USUHS from the National Institute on Drug Abuse (DA07110), and USUHS (RO8882).

Family History Study. The specific aims are (1) to compare the response to an acute alcohol challenge in individuals at low and high risk for substance abuse as defined by family history of alcoholism, (2) to compare the response to a stimulant drug (*d*-amphetamine) in the same groups, and (3) to examine the concordance of response to a stimulant and a sedative drug within subjects.

Drug Preference Study. The specific aims are (1) to test the hypothesis that individuals who prefer *d*-amphetamine over placebo will report more stimulant-like subjective effects, while individuals who prefer placebo will report other types of subjective effects, and (2) to test the hypothesis that individuals who prefer*d*-amphetamine over placebo will show behavioral and electrophysiological responses consistent with the stimulant-like subjective effects, while individuals who prefer placebo will have a different profile of behavioral and physiological effects.

Twin Study of Continuous Performance Test. This study will (1) permit estimates of the heritability of sustained attention, (2) examine developmental changes in that heritability, and (3) disentangle the effects of heredity and environment on the observed gender differences in the CPT.

B. Methods Employed

Family History Study. Individuals with (N=30) and without (N=30) a family history of alcoholism will be recruited to participate. A within-subjects design will be employed to examine concordance between stimulant and sedative drug response. Testing will consist of 5 sessions: a placebo session, two *d*-amphetamine, and two alcohol sessions. Response to drug will be measured using a battery of measures including resting EEG, event-related potentials, the Continuous Performance Task, physiological tremor and standing stability, and self-reported subjective effects.

Drug Preference Study. There will be two phases of this study: in the first phase, preference for *d*-amphetamine vs. placebo will be determine using a behavioral choice procedure. In the second phase, subjects who are designated as either Choosers or Nonchoosers will participate in three psychophysiology sessions. In one session they will receive placebo, in one session they will receive 5 mg and in one, 15 mg *d*-amphetamine. Their response to drug will be measured using the same battery of electrophysiological and behavioral measures as described in (1).

Twin Study of Continuous Performance Test. MZ (N=100) and DZ (N=100) twin pairs will be tested in 6 variants of the CPT (visual X, visual AX, degraded X, auditory tones, auditory "O", and degraded auditory "O"). Zygosity diagnosis will be based on questionnaire; blood typing will be used to resolve ambiguous diagnoses. When possible, access to obstetrical records will be obtained and the relationship of pre- and perinatal trauma to CPT performance and cotwin similarity will be quantified.

C. Major Findings

No subjects have been run yet in the family history study; 70 pairs of twins (ages 8-81) have been run in twin study of the CPT, and preliminary results are being analyzed at this time

In the Drug Preference Study, we have run 12 subjects through the behavioral choice procedure. Four have chosen *d*-amphetamine 4 or 5 times out of 5 (Choosers), 6 have chosen *d*-amphetamine 0 or 1 time out of 5 (Nonchoosers), and 2 have chosen *d*-amphetamine inconsistently. In addition, we have recruited an additional 40 potential subjects and are screening them now to determine whether they meet the inclusion criteria for the study. While we do not yet have a sufficient sample size to permit comparison of Choosers and Nonchoosers on demographic variables or subjective drug effects, we used repeated measures ANOVAs to test drug x time effects on our self-report measures in our sample of 12 subjects. These results reveal that, on the average, *d*-amphetamine is having the predicted effect, causing stimulant-like effects which peak variously at Hour 1 or Hour 3. An examination of individual differences in this stimulant-like effect as a function of Chooser vs. Nonchooser status awaits a larger sample size. As subjects are run in the electrophysiology phase of the study, we will test the hypothesis that Chooser status accounts for individual differences in behavioral and electrophysiological response to *d*-amphetamine.

D. Significance to Biomedical Research

Family History Study. A salient issue in the study of drugs of abuse is whether vulnerability to substance abuse is specific, increasing the risk of abusing specific drugs, or whether there is a common vulnerability across substance abuse disorders. This project focuses on drug response as a possible contributor to vulnerability to substance abuse and addresses the question of specificity. Responses to alcohol and *d*-amphetamine are being assessed with a battery of electrophysiological and behavioral measures. This study will contribute to our understanding of the role of differential drug response in accounting for differences in vulnerability to substance abuse and will address the question of whether a common biological mechanism underlies the stimulant actions of stimulant and sedative drugs.

Drug Preference Study. It has been shown that individuals vary in their preference for amphetamine and that those individuals who choose the drug over a placebo are those individuals who experience stimulant-like subjective effects of amphetamine. EEG measures have been found to be sensitive to amphetamine effects and to distinguish subgroups of subjects who experience stimulant and paradoxical depressant-like effects of amphetamine subjectively and electrophysiologically. The present study will compare the electrophysiological response to *d*-amphetamine in Choosers and Nonchoosers. By addressing the relationship between drug preference and drug effects, the research will provide information on individual differences in vulnerability to substance abuse.

Twin Study of Continuous Performance Test. The CPT provides a measure of sustained attention that has been used to study the attention deficit in schizophrenia and other disorders. Despite evidence that a vulnerability to many of these disorders may be genetically transmitted, there is little data bearing on the heritability of attention deficits. The twin study addresses this issue.

E. Proposed Course

We plan to run 30 family history positive and 30 family history negative subjects in our Family History Study. Phase I of the Drug Preference Study (the designation of individuals as Choosers or Nonchoosers) will continue until we reach our predetermined sample size (i.e., $N_{Choosers}=12$ and $N_{Nonchoosers}=12$). We require an additional 8 Choosers and an additional 6 Nonchoosers. Recruitment and screening are ongoing. Phase II of the preference study (the electrophysiology protocol) will be underway soon and will continue until completion. For the Twin Study of the CPT, we plan to continue recruiting twins until we reach the designated sample size. Additional twins will be recruited if the age range is not adequate to permit developmental-genetic analyses.

F. Publications

Gabbay FH. Behavior-genetic strategies in the study of emotion. Psychol Sci 1992;3: 50-55.

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

PROJECT NUMBER
Z01 MH 02404-06 LPP

PERIOD COVERED
October 1, 1991 to September 30, 1992

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: Allan F. Mirsky, Ph.D., Chief, LPP, NIMH
Co-PI: Bruno J. Anthony, Ph.D., Assistant Professor, Department of Psychiatry
University of Maryland, Baltimore County, Guest Researcher, LPP, NIMH
Others: Connie C. Duncan, Ph.D., Research Specialist, LPP, NIMH

COOPERATING UNITS (If any)
Department of Psychiatry, University of Maryland

LAB/BRANCH
Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
1.0	0.5	0.5

☒ (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 goal of this project is to examine dysfunction of preattentive and attentive regulatory mechanisms in children and infants who exhibit disturbance in the modulation of cognitive, affective, and social processes. Actions of these regulatory mechanisms are assessed through examination of steady-state autonomic and central nervous system activity and of the response of these systems to environmental events. Measurement of the blink reflex, heart rate, respiration, and task performance during rest and in response to simple two-stimulus paradigms permit assessment of the integration of different neural systems (sensory, motor and autonomic), preattentive inhibitory and excitatory effects on sensory processing, and different components of attention including intensity, maintenance, breadth (focus or selectivity), and resistance to distraction. Data collection has continued in a studies of regulatory functioning in groups of 7-to-12-year-olds with various neuropsychiatric disorder. Results of analyses of preattentive processing in a group of 30 children with Attention-deficit hyperactivity disorder (ADHD) indicate greater difficulties in brainstem-mediated regulation for ADHD without co-occurring conduct and aggressive problems.

Major Findings

We have completed data collection of a variety of measures of preattentive processing and have analyzed startle blink data from a group of children with Attention-deficit Hyperactivity Disorder (ADHD). If automatic processes lay the groundwork for further analyses by attentive processes, disruption of attention-demanding communicative, social, and cognitive activities seen in ADHD children may be secondary to a distortion of preattentive processes. Startle blink paradigms provide a means of tapping such processes. A brief prestimulus occurring from 20 to 400 ms prior to a startle-eliciting probe results in a marked inhibition of blink magnitude relative to the response to the probe alone. This phenomenon, termed prepulse inhibition (PPI), has been assumed to reflect a low-level, sensori-motor gating process. In contrast, longer duration prestimulation lasting until probe onset produces blink facilitation and appears to reflect an activation process that enhances sensory and motor processing.

Attempts to elucidate core deficits in ADHD have been hampered by sample heterogeneity. Recent research suggests that ADHD children with co-occurring disruptive disorders, characterized by difficulties with authority and aggression, may constitute a distinct subtype. Therefore, the present study examines inhibitory and excitatory blink modulation in groups of ADHD children differentiated by the presence of a disruptive disorder.

The subjects were 14 normal controls (11 M, 3 F), 15 ADHD children (11 M, 4 F), and 15 ADHD children who also met DSM-III-R criteria for Conduct Disorder or Oppositional Defiant Disorder. The groups did not differ in age (mean = 113 months), although the Controls achieved a somewhat higher Verbal IQ score. All children were medication-free for more than 24 hours prior to testing. Subjects completed eight, five-trial blocks, with each block containing one trial in each of five conditions: a probe alone condition consisting of a 50 ms burst of 102 dB(A) white noise; three inhibitory conditions in which a 50 ms, 1 kHz tone at 76 dB(A) preceded the probe by SOAs of 50 ms, 125 ms, or 250 ms; and, a facilitatory condition in which the prestimulus tone was continuously present for 4000 ms prior to probe onset. Reflex blink was measured from orbicularis oculi EMG recorded for 500 ms following probe onset on each trial.

Significant modulation effects were evident in the overall results, similar to the pattern seen in adult studies. Compared to the probe-alone response, blink magnitude was reliably inhibited in the 125 ms SOA condition and reliably facilitated by the continuous 4000 ms prestimulus. Also, blink latency was significantly shortened in the 50 ms and 4000 ms SOA conditions. Group comparisons revealed differences only between Controls and ADHD children without associated disruptive disorders. The "pure" ADHD group showed less magnitude facilitation in the 4000 ms condition and reduced PPI in the 125 ms condition.

These results suggest that a subgroup of children with relatively pure ADHD possess difficulties in basic regulatory processes most likely mediated at the brainstem level. The findings are consistent with other work indicating distinct subgroups of ADHD. Compared to those with co-occurring conduct and aggressive problems, "pure" ADHD children are likely to exhibit neuropsychologically-measured inattention, learning difficulties, and neuro-developmental delays.

Significance to Biomedical Research and to the Program of the Institute

The adequate assessment of attentive dysfunction and underlying preattentive dysfunction is critical for the understanding of the neuropsychiatric disorders of childhood and the prevention of later-occurring psychopathology and substance abuse. 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. 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. Over half of adolescents with Attention Deficit Disorder are referred to the courts for theft and truancy and one quarter are later diagnosed as possessing Anti-social Personality Disorder. Moreover, attention problems may represent a factor which is either central to, or increases the vulnerability of those children already at genetic risk for serious psychopathology such as schizophrenia.

Proposed Course

We plan to continue analyses of data obtained from the ADHD children as well as those with other disorders such as Tourette's Syndrome and Obsessive-Compulsive Disorders. In addition, we plan to relate these data to both the neuropsychological and event-related potential data collected on these subjects.

Publications

Anthony BJ. Mechanisms of selective processing in development: Evidence from studies of reflex modulation. In Jennings JR & Coles MGH eds. Handbook of Cognitive Psychophysiology. Sussex, England: John Wiley, 1991;576-620.

Anthony BJ, Friedman D. Development of processing control mechanisms: The interplay of subcortical and cortical components. In: Jennings JR & Coles MGH, eds. Handbook of Cognitive Psychophysiology. Sussex, England: John Wiley, 1991;657-683

Anthony BJ, Phillips S. Attention deficit hyperactivity disorder. In: McAnarney ER, Kreipe RE, Orr DT, Comerci GC eds. Textbook of adolescent medicine. Philadelphia, PA, Saunders, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00672-27 LSES

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Social Psychological Correlates of Occupational Conditions

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

PI:	C. Schooler	Acting Chief	LSES, NIMH
Others:			
	C. Schoenbach	Social Science Analyst	LSES, NIMH
	M. Kohn	Guest Researcher	LSES, NIMH
	P. Darby Lipman	IRTA Fellow	LSES, NIMH
	L. Caplan	Staff Fellow	LSES, NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.6	2.0	.6

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 year we continued an extended series of analyses of the psychological effects of occupational conditions previously collected by the Laboratory. In these complex analyses our aim is to model the effects of parents' social status, occupational conditions and parenting behavior on the intrafamilial transmission of intellectual flexibility, distress, and self-directed orientations and values.

We also made considerable progress in developing a new longitudinal study of the reciprocal effects of social environments and psychological functioning in older people. One major accomplishment was the relocation of 95% of the respondents of the LSES's 1974 survey, who will serve as the sample for the new study. We have also developed and pretested an initial version of our interview. This initial version gets information about the occupational conditions of those who are still employed, measures the nature of housework, voluntary and leisure time activities, and provides measures of the relevant psychological variables.

Project Description:

The study of the psychological effects of occupational conditions has been the central focus of the LSES's sociological and social psychological research program for more than two decades. The study was originally conceived in the early 1960s by Melvin Kohn and Carmi Schooler as a way of testing the hypothesis that the comparatively greater value that lower socioeconomic status parents place on conformity in their children and the comparatively greater value that higher status parents place on self-directedness is a function of the different requirements necessary to succeed in the jobs those parents are likely to hold.

During the planning phase the scope of that inquiry was enlarged in several important ways. We expanded our hypothesis to the more general one that social status differences in people's orientations toward themselves and their environments and even in the ways people think are a function of the nature and conditions of their work. To test this more general hypothesis, instead of comparing specific jobs, we conceived of a job in terms of a series of dimensions (e.g. closeness of supervision, routinization, substantive complexity [the three jointly seen as indicative of occupational self-direction], ownership, bureaucratization, position in the hierarchy, time pressure). We also expanded the psychological variables we examined beyond the realm of parental values to include values for oneself as well as psychological measures such as self-esteem, anxiety, authoritarianism and, perhaps most unusually for a survey of that time, intellectual flexibility.

The original 1964 sample of 3,101 men was representative of all men in the United States employed in civilian occupations. In a 1974 follow-up, a representative subsample of these men were re-interviewed, and their wives and children were interviewed for the first time. Analyzing the men's longitudinal data with the then newly developed technique of structural equation modeling, we found that jobs that facilitate occupational self-direction increase intellectual functioning and promote a self-directed orientation to self and to society. Further findings demonstrated that opportunities for exercising occupational self-direction -- especially for doing substantively complex work -- are to a large extent determined by a job's location in the social structure of society. Other results indicated that oppressive working conditions produce a sense of distress. In all of these findings there is the consistent implication that the principal process by which personality is affected is generalization from the lessons of the job to life off-the-job.

These findings about the effects of occupational conditions on men were replicated cross-culturally in studies carried out in collaboration with Japanese and Polish investigators and extended to

women in the U.S. and Japan. Analyses of the psychological consequences of the household work of American wives indicated that such work has generally the same psychological effects as work done for pay. Preliminary analyses suggest that the same is true for Japanese wives. Other findings strongly suggest that in both sexes complex self-directed work leads to intellectually demanding leisure-time activities.

Exploring whether a similar pattern of environmental effects occurs at earlier stages of life, we examined the data from the interviews of the respondents' children. Our analyses show that self-directed complex school work increases children's intellectual flexibility and self-directed orientations just as self-directed complex work affects their parents. Other analyses also provided evidence that exposure to a complex environment during childhood has effects on adult psychological functioning similar to the effects on adult functioning of exposure to environmentally complex occupational conditions during the middle of the life span.

The presently ongoing research on this project can be divided into two categories: I) Continued analyses of already collected occupation study data, with particular emphasis on the socio-environmental determinants of the intrafamilial transmission of cognitive and non-cognitive aspects of psychological functioning; II) Developing and carrying out a new follow-up survey of the respondents in the LSES occupation study to examine environmental effects on psychological functioning in older people.

I. Continued Analyses of Occupation Survey Data:

In an extended series of analyses, Kohn, Schooler and Schoenbach are modeling the relative effects of parents' background, social status and occupational self-direction on the transmission of intellectual flexibility, distress, and self-directed orientations and values from parents to their children. These models are complex in that they examine the network of causal paths, including potential reciprocal effects, through which parents' social background, occupational conditions and psychological characteristics may affect not only the parents', but their children's, psychological functioning. Preliminary findings indicate that, as hypothesized, parents' occupational self-direction increases the intellectual flexibility and the self-directedness of their children.

Schooler and Schoenbach are working on even more complex models for the U.S. families, which examine how parents' supportive behavior and controlling behavior affect children's psychological functioning. These models not only take into account family background characteristics, but also include parents' psychological characteristics and the possibility of reciprocal effects between the children's psychological characteristics and parental behavior; they have, therefore, proved very difficult to estimate. We are now in the process of developing separate models for boys and for girls.

II. Reciprocal Effects of Social Environments and Psychological Functioning in Older People:

Although we are clearly deeply involved in analyzing the data already in hand, we are very excited at the prospect of collecting new data. With strong encouragement and the high expectation of receiving very substantial support from the Behavioral and Social Research Program of the National Institute on Aging, we are far along in the planning of a longitudinal study of the reciprocal effects of social environments and psychological functioning in older people.

The empirical basis of this investigation would be a resurvey of the 1974 respondents. Through a contract let to the Equifax Co., and largely funded by NIA, we have now succeeded in relocating 650 of these households.

Among the issues of concern in planning the interview are:

1. Extending the theory of the psychological effects of complex environments to older adults by adding to the existing conceptualization and measurement of the complexity of older people's life settings.

2. Testing whether the causal connections among environmental complexity, self-directed orientation, and intellectual flexibility are the same for people when they are older adults as when they were younger.

3. Investigating the extent to which there are individual differences in what is an optimal level of environmental complexity.

4. Linking our measures empirically to other measures of intellectual functioning, thus expanding the aspects of such functioning that we measure.

5. Examining the significance of self-directed orientation in later life. Self-directedness has clearly positive connotations of control, self-efficacy, and individualism in early and middle life. But perhaps some degree of self-directedness is traded for safety and security among older adults.

6. Collecting detailed information on social networks and social support.

7. Because conditions of health take on an even greater significance in old age, multiple indicators of health status will also be included. We will try to explore possible reciprocal effects of health status, psychological functioning and socio-environmental conditions.

8. We are also adapting for inclusion items on role strain, stress and coping from an earlier LSES study by Pearlin and Schooler on the structure of coping. Because of the extensive data we will have on the respondents' personal histories, health status, social and physical environment and psychological functioning we should be able to

examine the determinants and effectiveness of the coping strategies they adopt.

This year, besides relocating 95% of our 1974 respondents, we have developed and successfully pretested an initial version of our interview, which deals with all of these issues. This initial version also gets information about the occupational conditions of those who are still employed, measures the nature of housework, voluntary and leisure time activities and provides measures of the relevant psychological variables. In addition, we are experimenting on the best way to have respondents recall and provide information about what has happened in their lives since we last saw them. On the basis of our progress in developing this new study we are now in the process of seeking financial support from NIA for the full implementation of our proposed study.

Significance of the Research:

The availability of an extensive set of social, psychological, environmental and behavioral data on children and both of their parents provides a rare opportunity for examining the mechanisms through which both psychological functioning and personal and social values are transmitted.

The possibility of getting long range longitudinal data (up to thirty years for the men in our sample, up to twenty years for the women) provides a unusual opportunity to bring several factors to bear on issues of substantive and practical importance involving social and psychological determinants of effective functioning in later life: the unique body of data that the LSES has already collected, the expertise and interest in the aging process of current Laboratory members, and the Laboratory's well recognized methodological sophistication in survey analysis and design.

Proposed Course of Research:

While we will continue with our analyses of the determinants the intrafamilial transmission of psychological functioning and social and personal values, we are eagerly anticipating the data collection and data analysis phases of our new project on the socio-environmental determinants of psychological functioning in the elderly.

Publications:

Melvin L. Kohn and Carrie Schoenbach. Social Stratification, Parent's Values and Children's Values: A Multinational Assessment. In D. Krebs and P. Schmidt (Eds.) The Relevance of Attitude Measurement in the Social Sciences. Heidelberg: Springer, 1992.

Carmi Schooler, Child Rearing and Child Behavior in the United States-Retrospective Assessment and Further Findings.
In D. Shwalb and B. Shwalb (eds.). Japanese Child Development: Classic Studies, Retrospects and Prospects. New York NY: Guilford Press. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00680-09 LSES

PERIOD COVERED

October 1, 1991 to September 30, 1992

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)*

PI: E. Liebow Guest Researcher LSES, NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER
1.0	1.0	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 overall objective of this exploratory, participant observation study was to develop a detailed picture of the day-to-day lives of homeless women. Specifically, the intent was to track the day-to-day experiences of homeless women over time and to analyze the ways in which these experiences affect their mental and emotional lives and the life course of their homelessness. Field work was carried out over more than three years, focusing on three night shelters and a day shelter in Montgomery County, Maryland.

Project Description:

Eight 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 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 nevertheless.

In November, 1984, Liebow began collecting data as a participant observer in three night shelters and a day shelter for homeless women in Rockville. Liebow followed the women intensively for three years and intermittently thereafter while writing up his experiences.

In final form, the main question asked by the research (developed in the course of writing up the material) is how the women remain fully human in the face of unremitting inhuman living conditions. An introductory chapter describes the people and the settings. Part I is titled Problems in Living. It consists of four chapters, each of which delineates a major problem area faced by homeless women: the routine problems of day to day living; problems with work and jobs; family; and last, problems with shelter staff and social agencies.

Part II is titled Making It: Body and Soul and consists of three chapters that focus on the physical and spiritual resources of the women: community supports, friends, religion, inner resources, and group solidarity.

Part III consists of brief, (4-5 page) life-histories of some 20 of the women and a section called Where are They Now? which answers that question for about a dozen women. The Appendix has two parts: a section on Research Methods, and a brief historical essay on attempts to develop a statistical picture of homelessness.

Significance of the Research:

The main value of this study lies in the detailed description of the individual and social lives of homeless women and of their problems in living as seen by both the women themselves and by an outside (but nearby) observer. These descriptions--developed over several years of close, first-hand observation--may help us to identify the many different needs of homeless women, to put them in priority order, and to gain a

better understanding of the physical, mental, and emotional resources the women themselves bring to homelessness. With luck, studies such as this one may help us to understand who becomes homeless, why they become homeless, and what we can do about it.

Proposed Course of Research:

This study is essentially complete and the resultant book accepted for publication.

Publications:

Liebow, E. Tell Them Who I Am: A Study of Homeless Women. New York: The Free Press, Scheduled publication in Spring, 1993.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00682-06 LSES

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Others:

C. Schooler, Acting Chief LSES, NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL	OTHER:
1.6	.6	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 transfer-appropriate model of memory, relying on the distinction between episodic and abstraction-based processing has been developed to account for the differential efficacy of analogical and other schematic retrieval aids for 1) individuals who have engaged in either simple or complex encoding of the material, and 2) younger and older adults. In general, such aids are beneficial for people who have engaged in previous complex encoding, or for younger adults. In contrast, they do not help, and may impair, the performance of those who have engaged in simple encoding, or of older adults. Recent studies have examined text comprehension processes using this model. In addition, the approach is being extended to the investigation of autobiographical memory and memory for public events.

Project Description:

In previous research conducted by this laboratory, Kohn & Schooler (e.g., Kohn & Schooler, 1983) found that people who were employed in more complex occupations demonstrated greater flexibility of thought even in tasks and contexts that were not job-related. They proposed that the type of thinking encouraged by occupational conditions generalized to thinking in other settings. Much of the work described below has grown out of an attempt to identify more precisely the psychological mechanisms responsible for this "generalization". In particular, we have hypothesized that complex environments encourage people to engage in "abstraction-based processing", i.e., to form psychological representations of their environments that are based on rules, features, or schemata that have been abstracted from events occurring in those environments. Alternatively, we propose that simple environments encourage people to engage in "episode-based processing", i.e., to form relatively unanalyzed holistic representations of those events themselves.

In a series of studies, we have tested these hypotheses as part of a larger model based on the principle of transfer-appropriate processing. Models of transfer-appropriate processing posit that memory retrieval is optimized when the processing occurring at the time of retrieval is the same type of processing that occurred at the time the relevant information was stored in memory. In this project, we have tested the hypothesis that learning involving the abstraction of information common to two domains (e.g., analogy-based learning) should be maximized when processing of that material has been complex; in contrast, we have hypothesized that learning which does not require such abstraction should be maximized when processing has been simple. To date, we have found support for these hypotheses using two major paradigms: computer learning and text comprehension. In addition, we have found that younger adults are more likely to benefit from abstraction-based learning experiences than are older adults. In the last year, we have completed the fifth text comprehension study in the series, and have begun to collect data for two experiments using new paradigms.

In the most recent text comprehension experiment, we have tested, compared, and integrated the predictions of our model and those derived from a major theory of analogy-based learning and memory. In this experiment, we asked subjects to read a test passage after they had read and encoded two source passages. We examined the effects of source passage encoding complexity and type of title provided in the test passage (no title, a title pointing out the analogy, and a title explaining the analogy). In addition, we asked half of our subjects to compare the source passages before reading the test passage. This experiment

yielded the expected interaction between encoding complexity and title, and failed to support the results of the alternate theory.

We have also been extending our theoretical approach to investigations of memory for real-life events, by running two experiments relevant to the planned third wave of the LSES's occupation study (see project entitled, "Social Psychological Correlates of Occupational Position). In the first, conducted with Dr. Lipman, we have begun collecting pilot data on people's memory for public events that have occurred since 1964. Half of the events are political in nature, and half are apolitical. Subjects are tested under one of three retrieval aid conditions: 1) control, 2) a timeline consisting merely of a list of the years from 1964 through 1990, and 3) a timeline consisting of years, with every Presidential election year accompanied by information about who won that year's election. Consistent with our earlier results, we expect that subjects who report being involved in political activities (i.e., whose political activities have been complex) will benefit more from the "Presidential" timeline than those who are not - particularly for the political events. We also expect that younger subjects will benefit more from the timelines than will older subjects. If the results of this pilot are promising, the experiment will be included in the interview to be used in the next wave of the occupation study.

The second experiment has grown from our need to develop methods of collecting information about people's life events over the 18 years that will have elapsed since these respondents were last interviewed. When asking people to recall such events, the series of questions can be structured by event type (e.g., illnesses, births) or by time period (i.e., year). This issue has theoretical, as well as practical implications. Several approaches to autobiographical memory suggest that people's memory for life events is structured as a narrative, with causal or thematic links among related events. If this is the case, then the time-based calendar should be more accurate than the event-based one. We have developed two forms of a life event history calendar corresponding to these two organizational principles, and are completing data collection in an experiment testing their efficacy.

Significance of the Research

These findings are significant for theories of memory and reasoning for several reasons. First, this work extends the theory of transfer-appropriate processing into new aspects of learning and memory. Second, our findings regarding the effects of age on the usefulness of abstract conceptual learning aids shed light on age-related changes in cognitive processes. Finally, the extension of our work into "everyday memory" will enable us to find out whether our laboratory findings can be safely generalized to memory for material experienced in natural settings.

Proposed Course of Research:

One of the more general challenges remaining for future research is to further specify the distinction between abstraction-based and episode-based processing. Currently, there is a host of similar distinctions in the cognitive psychology literature that seem to address a common dichotomy: implicit/explicit memory, exemplar/feature memory models, nonanalytic/analytic models of memory and perception, analogical/schematic approaches to problem-solving and transfer, textbase/situation models as alternative representations of textually presented materials, and data-driven/conceptual processing. In all of these distinctions, the first "type" of representation or processing listed corresponds to what we refer to as episode-based processing, and the second corresponds to abstraction-based processing. Nevertheless, the parallels are rough: for example, it is currently unclear whether implicit memory is necessarily abstraction-based, and explicit memory necessarily episode-based. An important next step in this project is to: 1) delineate the relationship between our distinction and others, 2) determine whether there does exist an underlying core of commonality among these distinctions, and 3) determine, if such a core exists, whether one of the existing conceptualizations corresponds most completely to that core.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00683-05 LSES

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Study of Social and Cognitive Aspects of Schizophrenia

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

PI: C. Schooler Acting Chief LSES, NIMH

OTHER:

Bruce Roberts, Social Science Analyst LSES, NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.9	1.3	1.6

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 program consists of a series of interconnected experiments, carried out on the same subjects, that examine the nature of four frequently cited psychological abnormalities in schizophrenia: (I) an inability to focus attention on task relevant aspects of the environment (II) anomalies in smooth pursuit eye movements (III) prolonged reaction times irrespective of temporal uncertainty or predictability of the forthcoming event and (IV) severe social dysfunctions as manifested by tendencies toward social withdrawal and cognitive disruption when dealing with social phenomena.

Project description:

This research program applies an experimental psychological approach to the study of ocular, cognitive and social interference in schizophrenia. The psychological nature of our approach is apparent in two basic ways: 1) Experiments that have been designed to study the psychological functioning of normal individuals have been adopted in ways that we believe will lead to more precise delineation of differences between schizophrenics and normals. Such delineation should help locate and specify those neuropsychological dysfunctions characteristic of schizophrenia. 2) Several of the experiments are explicitly designed to explore the psychological ramifications of phenomena that are generally seen merely as markers of neurological dysfunction.

Although these abnormalities are not all necessarily related, by examining them on the same subjects we hope to test a set of hypotheses that predict the existence of empirical relationships among them stemming from their being, at least in part, manifest indicators of more basic disturbances in schizophrenics' psychological and prefrontal functioning.

The first set of experiments aims at elucidating the nature of difficulties schizophrenics seem to have focusing attention on task-relevant aspects of their environment while ignoring other aspects. Being able to focus attention in this way is a necessary prerequisite for all goal-directed behavior. Schizophrenics' behavioral and cognitive dysfunctions have often been characterized as reflecting basic difficulties in selective attention and in inhibiting the processing of task-irrelevant features or objects.

Most past studies have dealt primarily with deficits in processing the to-be-attended stimuli in a given task. In contrast, the proposed studies focus on how schizophrenics process those environmental features which should be ignored while attention is directed towards a predefined set of task-relevant targets. In particular, we examine the extent to which schizophrenics can actively inhibit the processing of distractors. Finding a distinctive pattern in schizophrenics of such difficulties in inhibiting responses to task-irrelevant stimuli would be relevant to several alternate but not necessarily incompatible hypothesis about psychoneurological dysfunction in schizophrenia: 1) inefficient activation of prefrontal cortical regions 2) differences in the characteristics of their neural traces (e.g., On the one hand, such traces "persist longer and at higher magnitudes than normal" according to Maher et al, on the other, they are modeled as having lower gain in Cohen and Servan-Schreiber's parallel distributed processing theory of schizophrenia) 3) dysfunction in

neural inhibiting processes.

Included among the experiments we are carrying out to explore the pattern of schizophrenics' difficulties in inhibiting inappropriate responses are procedures testing whether and how a) the Stroop effect, b) distractibility in a version of the Posner et al. directional response task, and c) positive and negative priming are more pronounced in schizophrenics than in normals. We are also testing whether the time course of the interference or the facilitation due to the accessory stimuli is different in schizophrenics than in others.

In another attempt to specify more clearly how schizophrenics handle to-be-ignored features or objects, our second set of experiments examines the extent to which such difficulties in inhibiting the processing of distracting stimuli explain the high level of the saccadic intrusions during smooth pursuit eye movements. So far these anomalies have generally been studied with the goal of establishing them as genetic markers for schizophrenia. Consequently, they have generally been considered as if they were an oculo-motor abnormality sui generis, functionally independent of the psychological, largely prefrontally determined processes implicated in performing the task. However, eye movement abnormalities including abnormalities in smooth pursuit and in memory guided saccades and difficulties with tasks such as the Wisconsin Card Sort and Stroop have been linked to neural pathways involving various areas of frontal cortex.

There seem to be various possible ways that such eye movement dysfunction may be neuroanatomically or neuropharmacologically linked to processes underlying the difficulty schizophrenics seem to have in inhibiting responses to inappropriate stimuli. Consequently, one of our research goals is to collect information that allows us to infer the degree to which smooth pursuit deviancies constitute a specific ocular-motor abnormality or whether they can be better understood as a systematic side effect of much more general difficulties in (1) inhibiting the processing of distracting information, and (2) programming motor responses that take advantage of the temporal redundancy in the target's rhythmic movements, both of may well be linked to prefrontal functioning.

The criterion variables will be the main forms of SPEM deviancies (e.g. number of catch-up saccades vs. number of saccadic intrusions, gain in periods of smooth pursuit, amplitudes of saccades). We have already begun work with Daniel Hommer of the University of Washington on the development of structural equation measurement models of SPEM abnormality, using data he has collected. He is fortunately also cooperating with us in applying his sophisticated SPEM data editing and reduction techniques to the SPEM data we are collecting.

To the extent that SPEM abnormalities can be predicted on the basis of measures derived from the above mentioned tasks, these abnormalities may not reflect specific anomalies in the oculo-motor pursuit system, but rather much more general abnormalities of schizophrenics in attention (inability to focus, i.e., to inhibit shifts of attention to task-irrelevant stimuli) or temporal motor-programming, both of which may well be linked to prefrontal functioning.

Difficulties of the latter sort are the focus of our third set of experiments. These experiments examine the apparent problems schizophrenics have in taking advantage of temporal redundancy in programming certain motor sequences. Such difficulties have, for example, been observed in schizophrenics not taking advantage of regularities in preparatory intervals in reaction time trials . Difficulties in the temporal organization of motor responses are also seen in their inability to adjust their speed of tapping to some external rhythm or to reproduce movement sequences that have been found in studies by Luria to be particularly difficult to copy for frontal lobe patients.

Among our experiments testing these difficulties are a visual parallel of the manual reaction time cross-over task, a replication of the original manual task, and various tests of the temporal programming of motor sequences and the reproduction of rhythms involving both visual and motor stimuli and interdomain transfer between the two modalities.

Our fourth set of experiments is a pair examining the nature of the interference in schizophrenics' cognitive functioning when they deal with social phenomena. The first tests whether social stimuli are particularly disruptive of the schizophrenic's ability to pay attention to task-relevant stimuli; the second tests whether this disruption is due to the specifically social nature of the stimuli or to the relatively complex nature that such stimuli frequently have.

In the first experiment, which involves dual task performance with social and non-social word stimuli, subjects are required to listen to verbs and to rate how pleasant or unpleasant they find the activity described by the verb, while carrying out our standard SPEM task. Comparisons will be made of their performance during trials involving social as opposed to non- social stimuli and pleasant vs. unpleasant word ratings.

The second social-psychological experiment examines a problem raised by the long series of experimental, observational and therapeutic studies that have consistently shown that, not only are schizophrenics prone to avoid social interaction, but they show a decrement in functioning as the intensity of social interaction increases. The reasons for such social dysfunction,

however, remain unknown. Although it is possible that this disruption of functioning in social circumstances may result from the specifically human characteristics (e.g., their level of expressed emotion) of the others in the situation, it is also possible that this disruption may be primarily of a cognitive, rather than a social nature.

Our experiment tests the performance of schizophrenics in social and non-social situations that are equated in their degree of cognitive complexity. The study takes advantage of the computer's ability to both present stimuli and evaluate response accuracy to test whether schizophrenics' performance on a perceptual task is more disrupted when performance accuracy feedback is presented by a person than by a computer. This test is carried out in a procedure in which every other parameter of the situation, including its cognitive complexity, is the same. It consists of identifying the quadrant of the presentation screen in which a target stimulus appears. The dependent variable is the speed of presentation at which such judgments can be made accurately. The major modification is that in the social feedback condition, accuracy feedback is given personally by the experimenter rather than impersonally by the experimental equipment, as it was in the original study, and as it will be in the non-social feedback condition of the present study.

All subjects are screened on the SCID. We also have coded a full social and psychiatric history for each. All subjects are also rated on the BPRS, the PSAS, the Maine Scale for Paranoid and Nonparanoid Schizophrenia, the Schedule for Deficit Syndrome, the UCLA Social Attainment Scale and the Neurological Evaluation Scale. Among the psychological tests the subjects receive are the: Proverbs Test, California Verbal Learning Test, WAIS Vocabulary and Digit Span, Wisconsin Card Sort and the Tower of London puzzle.

Completing our full experimental series takes about 25 hours.
As of now we have run over 65 subjects through our basic experimental protocol. These subjects have come from the NIMH Neuropsychiatric Hospital at Saint Elizabeths, from the inpatient wards at St. Elizabeths and from the Area D outpatient center.

Although this LSES experimental research program on schizophrenia also includes various ancillary experiments, it is worth noting that this experimental psychopathology research focuses on the same questions as the Laboratory's sociological research-- How environmental complexity, intellectual functioning and the social environment of individuals are causally related?

Significance of the Research:

Although the LSES had a long history of both sociological and experimental psychological research on psychopathology,

particularly schizophrenia, in recent decades we did no new research in this area. Some of the reasons for turning away from psychopathology research lay in the apparent absence of research and data analytic techniques capable of dealing with the complex nature of the problems which concerned us. Other reasons lay in the absence of possible genetic markers and plausible neurobiological models to help explain the tremendous variance our research found in the schizophrenics' responses to cognitive and social-psychological stimuli. The absence of such markers and models also seemed to severely hinder the development of appropriately sophisticated models of how neurobiological and social levels of phenomena interact to produce psychopathology.

Several events within the last decade have substantially changed this picture. Not only have structural equation modeling and related maximum likelihood statistical techniques become more widely accepted by researchers in the area, but new sociological and biological findings have raised important research questions. From the Laboratory's perspective the two most exciting of the biological findings are the interrelated ones of the evidence that smooth pursuit eye movement (SPEM) irregularities plausibly represent a genetic marker for schizophrenia and the parallel accumulation of evidence of abnormal functioning in portions of at least some schizophrenics' frontal lobes and connected brain areas. The present research program represents an exciting opportunity to combine the Laboratory's expertise in the areas of experimental psychopathology, social behavior and causal modeling to test hypotheses about the pattern and possible biological and social causes of schizophrenic dysfunction.

Proposed Course of Research:

Present plans call for testing at least 70 schizophrenics (more may be required for the structural equation analysis we hope to do), 35 non-schizophrenics from the same facilities from which the schizophrenics are drawn and 35 normal controls. The non-schizophrenics will be matched with the schizophrenics on age, race and parents educational level.

Several types of statistical analyses will be used: (1) Analyses of Variance and Covariance which treat each experimental procedure separately and which compare the performance of the schizophrenics to the psychiatric and normal control groups. (2) correlational analyses within the schizophrenic sample that examine the relationships of symptom patterns to responses to the different experimental conditions; (3) structural equation modeling that will test hypotheses that the schizophrenics' responses to the various experimental conditions are determined by a limited set of anomalies in their functioning (e.g., relative inability to inhibit the processing of task irrelevant information, unusual persistence of neural traces, difficulty in responding in ways that take advantage of regularities or other sources or information about relevant stimuli); and (4) if the existence of such latent factors is confirmed, linear structural

equation analyses will be carried out in an effort to model the causal relationships among these factors and between these factors and what other information might be made available to us about the patients' neuro-pathology, neurophysiology and psychopathology.

Publications:

Carmi Schooler, Statistical and Causal Interaction in the Diagnosis and Outcome of Depression. In House, J., Blazer, D. and Schaie, K.W. (Eds.), Health Behavior and Health Outcomes. Hillsdale, N.J.: Erlbaum, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00684-05 LSES

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

The Representation of Semantic Categories

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

PI: L. Caplan Staff Fellow LSES, NIMH

COOPERATING UNITS *(if any)*

National Institute on Aging

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.3	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.)*

A model of semantic category representation is being developed to account for a variety of categorization phenomena. In previous work, we demonstrated that categories represented primarily by intrinsic features (i.e., features true of an object in isolation) and those represented primarily by extrinsic features (i.e., relations among entities) differ in structure. Recently, consistent with the model, we have demonstrated that context effects and inter-subject disagreement among subjects in categorization judgments are greater for extrinsically represented than for intrinsically represented categories.

Project Description:

In this project, we are refining and testing a model of semantic category representation of natural language categories originally developed by Leslie Caplan in collaboration with Dr. Robin Barr from the National Institute on Aging. Semantic categories are critical to many issues in cognitive psychology, such as language, memory, problem-solving, and decision-making. In the model, we distinguish between two kinds of features which may comprise a category representation: intrinsic (i.e., those features represented as true of an object in isolation) and extrinsic features (i.e., those features represented as relations among entities). In past work, we demonstrated that semantic categories differ in the degree to which their representations rely on these two types of feature, resulting in inter-category differences in structure and category-based reasoning. More recently, we have modified the model to include inter-category differences in the degree to which such features are represented as necessary for category membership.

In the last year, we have focused on how membership in intrinsically and extrinsically represented categories differs as a function of context. According to the model, members of extrinsically represented categories should be more likely to change membership status with changes in context than should members of intrinsically represented categories. In addition, judgments of typicality should be more context-sensitive than category membership judgments. In a paper currently in preparation, we include data from a previous experiment of context effects on categorization, as well as data from the two studies described below.

In one experiment, we have recently completed data collection from a replication of an earlier study of context effects, this time including reaction time measures as well as membership and typicality ratings as dependent variables. We have replicated our previous major findings from membership and typicality ratings, and these results provide more support for the model. In addition, preliminary analyses of our reaction time data have yielded results that are largely consistent with predictions derived from the model.

In the second experiment, we have hypothesized that differences in personal histories should lead to inter-individual differences in the structure of semantic categories and available retrieval cues (i.e., retrieval contexts). Therefore, we expected that individuals' category judgments would differ more for extrinsically represented categories than for intrinsically represented categories, and that such differences would be more apparent for typicality than for membership judgments. Both of these hypotheses were confirmed.

Significance of the Research

This model of semantic category representation has been very successful both in accounting for results obtained by other researchers in the field, and in predicting new categorization phenomena. These findings have implications for how people use natural language categories in everyday reasoning tasks. They may also provide some hypotheses about the nature of disruptions and deficits in semantic memory frequently observed in individuals who have experienced various types of brain damage.

Proposed Course of Research

Currently, two papers on the representation of semantic relations (a closely related topic) are under review. As described above, a paper investigating contexts effects on categorization is in preparation, and the next experiment in that series is in preparation.

Publications:
None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02495-03 LSES

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Memory Functioning in Normal and Neurologically-Impaired Individuals

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

PI: C. Schooler Acting Chief LSES, NIMH

Others:

P.D. Lipman IRTA Fellow LSES, NIMH

C. Schoenbach Social Science Analyst LSES, NIMH

COOPERATING UNITS *(if any)*

Cognitive Neuroscience Section, NINDS, NIH (J. Grafman); VHIS, Walter Reed Army Medical Center (K. Schwab).

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL:	OTHER:
0.3	0.3	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 year the Laboratory continued its series of analyses that use linear structural equation techniques to examine the relationship between memory function and different types of penetrating missile head wounds. This research is based on work the Laboratory is doing using similar analytical techniques to investigate the architecture of memory in normal individuals.

Project Description:

This year the Laboratory continued its series of analyses examining the relationship between memory function and different types of penetrating missile head wound. This research is based on work the Laboratory had done earlier on the architecture of memory in normal individuals. In this earlier research, Carmi Schooler, Paula Darby Lipman, Douglas Herrmann and Carrie Schoenbach, in collaboration with Jordan Grafman of NINDS, used confirmatory factor analysis on data from normal Vietnam War veterans.

Significance of the Research:

This research should help us understand the nature of cognitive deficits resulting from neurological damage, by doing so it should also provide valuable insights into normal memory functioning.

Proposed Course of Research:

We plan to continue our examination of the data from the brain-injured Vietnam veterans, and to link the nature of the cognitive impairment with the nature of the head injury. Once these analyses are conducted, the results will be prepared for publication.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02496-03 LSES

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Age Differences in Large-Scale Spatial Processing

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

PI: P.D. Lipman IRTA Fellow LSES, NIMH

Others:

L. Caplan Staff Fellow LSES, NIMH

COOPERATING UNITS *(if any)*

Cognitive Neurosciences Section, NINDS

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER
1.1	0.7	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.)*

This project investigating large-scale spatial encoding (e.g., of visual geographical route and configural information), allows us to systematically examine factors influencing memory for landmarks (episodic, scene-based knowledge), and memory for routes and configurations (abstract, layout-based knowledge). We are continuing to explore whether cognitive representation and retrieval of sequentially ordered route scenes and other real-world spatial environments are influenced by experimental manipulations (particularly the presentation of map-like stimuli), and the relationship between performance on various measures of spatial memory and subject factors (age, gender, and performance on selected standardized measures of spatial abilities).

PHS 6040 (Rev. 5/92)

Project Description:

This research links previous work on age differences in macro-spatial cognition conducted by Lipman, and research on complexity and cognitive functioning conducted in the lab by Caplan and Schooler. Using the model proposed by Caplan and Schooler (see project titled "Environmental Determinants of Cognitive Functioning"), we have completed a study which followed up on Lipman's earlier findings (1991) concerning age-related differences in acquisition of route information. In this study currently in press (Lipman and Caplan, 1992), we continued to examine changes with age in spatial information processing, as well as predictions based on the Caplan and Schooler model. We tested two age groups (25-40 years, and 60-75 years), and presented two sequences of slides depicting two overlapping routes. We varied encoding instructions and the presence or absence of a schematic diagram of the routes. Based on the results, we suggested a distinction between scene-based and layout-based representation. The former underlies memory for landmarks, whereas layout-based representation is essential for configural memory. In particular, we found that layout representation was related to both age and the presentation of the diagram, such that the diagram improved memory for turns and configurations for younger subjects, but impaired performance for older subjects. These results are similar to those obtained by Caplan and Schooler (again, see "Environmental Determinants of Cognitive Functioning" project), and suggests that older adults have difficulty engaging in the elaborative activity that is required in the use of diagrams.

Data analysis is underway on another experiment in which we tested two alternative hypotheses which might explain the interaction between age and diagram obtained in the Lipman and Caplan (1992) study. According to the first hypothesis, cognitive capacity limitations make it difficult for older adults to use maps, diagrams, or other learning aids, because the elaborative activity required for the use of such stimuli is constrained by resource limitations. According to the second hypothesis, older subjects in the earlier study had difficulty using the diagram because it did not contain enough detail to be meaningful, i.e., to be interpretable in terms of existing cognitive structures. Therefore, we are currently exploring our data for the effects of three variables on memory performance of younger and older adults: whether or not a "map" is provided, whether subjects are told that the "map" is a diagram or a map, and whether or not landmarks are labelled on the map. If the hypothesis about limited cognitive resources is correct, then any kind of map or diagram should impair the performance of older adults. In contrast, if the hypothesis about meaningfulness is correct, then providing map-like detail, or labeling a map as such, should increase the beneficial effects of map-like stimuli for older adults.

In addition, Lipman and Caplan, in collaboration with Jordan Grafman, Chief of the Cognitive Neuroscience Section, NINDS, are in the early phase of development of a study of people's understanding and memory of the spatial layout of the NIH campus. This study will allow us to investigate people's representations of a real spatial environment, rather than one depicted and learned through a slide presentation. At the same time, we will be able to examine factors which may influence employees' spatial memory such as age, length of employment, location of office building, navigation behavior and habits, and individual differences in spatial abilities. In particular, we hope to investigate whether there are independent effects of age and amount of experience on abstraction-based processing of large-scale spatial information, and whether individuals of different occupational and psychometric profiles demonstrate reliable differences in their representations. Prior to initiating the full-scale study, we are running a pilot study to determine the most frequented buildings and paths on campus.

Significance Of the Research:

This research has the potential to expand our understanding of learning and problem solving in an everyday context -- real-world spatial environments -- with a specific focus on the impact of changes with age on large-scale spatial processing. This research has both practical and theoretical importance. Findings from this project may shed light on possible age differences in the use of maps in way-finding and spatial memory -- an area in which relatively little research has been conducted. Its theoretical contribution is the integration of issues pertaining to age-related changes in information processing, in particular older people's capacity to make use of learning aids such as diagrams, into the literature on spatial cognition.

Proposed Course of Research:

Data analysis is underway for the followup to the Lipman and Caplan (1992) study. Also, as described above, a study of memory for the spatial layout of NIH is currently being planned.

Publications:

Lipman, P. D., & Caplan, L. J. (1992). Adult age differences in memory for routes: Effects of instruction and spatial diagram. Psychology and Aging, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02566-01 LSES

PERIOD COVERED

October 1, 1991 to January 10, 1992

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

Abstraction, Aging and Environmental Complexity

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

PI: J. Lee IRTA Fellow LSES, NIMH

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.0	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.)*

Since Dr. Lee has left the LSES this project has been closed. The relevant abstraction research is now included in Project Z01 MH 00672-27.

PHS 6040 (Rev. 5/92)

Project Description:

The Laboratory of Socio-environmental Studies has a longstanding interest in the social bases of intellectual functioning. Over the years and across many research projects a generalized "theory of psychological effects of complex environments" has been developed to account for a recurring pattern of empirical findings. Its thesis is that environmental complexity--in school, at work, and in the home--facilitates higher levels of intellectual flexibility.

Jason Lee joined the Laboratory in January of 1991 to do further research in this area. Coincident with his arrival his book on abstraction and aging was published by Springer-Verlag. In this book empirical analyses of relationships between social experience and abstraction across the life course are organized using the theory of the complexity of social environments discussed above. Lee's work followed up on his earlier research by a) further developing the conceptualization and measurement of abstraction, and b) extending the conception of environmental complexity to encompass elder's lives.

The goal of Lee's research on abstraction was to produce a measure of intellectual functioning which is conceptually clear, informed by cognitive psychological theory and research, and suitable for use in survey research (i.e. with a heterogeneous, randomly selected sample of respondents).

Significance of Research:

Abstraction is a basic facet of intellectual functioning. Gaining a more definite understand of it should lead to greater comprehension of other basic cognitive processes, such as categorizing, generalizing, and problem solving.

Proposed Course of Research:

Since Dr. Lee has left the LSES this project has been terminated as of January, 1992. The relevant abstraction research is now included in Project Z01 MH 00672-27.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02610-01 LSES

PERIOD COVERED

October 29, 1991 to September 30, 1992

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

Socioeconomic Status, Schizophrenia and Psychotic Symptomatology.

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

PI: C. Muntaner Visiting Associate LSES, NIMH

COOPERATING UNITS *(if any)*

Epidemiology Genetics Program. Dept. of Psychiatry. School of Medicine. Johns Hopkins University.

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	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.)*

This study examines questions about the relationship between social stratification and social class, on the one hand, and the etiology and symptomatology of psychiatric illness, on the other. Participants are drawn from a large sample of psychotic disorder patients (Schizophrenia, Bipolar Disorder, Other DSM-III Axis I disorders). We use structural equation modeling (LISREL) for determining the pathways by means of which socioeconomic status of the patients' family of origin is associated with the patients' socioeconomic status at the time of admission into a psychiatric facility. Differences in the fit of the models among specific samples of psychotic disorders (e.g., Schizophrenia, Bipolar Disorder) are also investigated.

A second aim of this project is the relationship between social stratification and the behavior of psychotic patients (e.g., aggressive behaviors). Methods involved in these analyses include linear and logistic regression modeling.

Project Description

Although the neo-Kraepelian research of the seventies and eighties seemed to have concluded that the effect of social structure on psychiatric disorders is very limited, new attempts to investigate this central problem of psychiatry research seem to indicate that such conclusions were premature (e.g., Dohrenwend et al., 1992; Fox , 1988; Link et al., 1986). The present study contributes to the advancement of our knowledge in this area by providing an improved assessment of DSM-III psychotic disorders and more sophisticated methods of analysis of "drift" data (e.g., structural equation modeling, log-linear analysis).

The project is based on a large sample of psychotic admissions to the inpatient psychiatric services in the Baltimore/Washington Area. All inpatient admissions with psychotic symptoms to fifteen hospitals between 1983 and 1989 were systematically screened. The diagnostic and sociological heterogeneity of the sample of psychotic patients was ensured by sampling different inpatient facilities: State mental hospitals (Spring Grove, Balto County; Springfield, Carroll County; Crownsville, Ann Arundel County; W.P. Carter CTR, Balto City; Highland Health, Balto City), university hospitals (Hopkins and University of Maryland, Balto City), private mental hospitals (Sheppard Pratt, Balto County; Taylor Manor, Howard County), and seven community general hospitals with psychiatric wards (Franklin Square, Balto County, FSK Medical Center, Balto City; North Charles, Balto City; Prince Georges Hospital, Prince Georges County; Montgomery General, Montgomery county; Ann Arundel General, Ann Arundel County; and Sinai Hospital, Balto County). Response rates as well as differences between participants and non-participants have already been reported. Participants were administered a version of the Diagnostic Interview Schedule, modified to improve its assessment of psychotic symptoms. The validity and reliability of diagnostic procedures has already been reported in previous publications (e.g., Pulver et al., 1989). Socioeconomic status (SES) of the patients' family of origin and the patient's own SES at the time of hospital admission were obtained through survey questions on occupation coded according to the census classification of industries and occupations.

A **first study** has examined the differences in SES between patients with Schizophrenia and Bipolar disorder at the time of first admission to a psychiatric facility (Muntaner et al., in revision). Using a logistic regression analysis we showed that Schizophrenic patients "drifted" to lower SES occupations than Bipolar patients, after adjusting for a number of confounders, including family SES. Furthermore, those low SES occupations were characterized by repetitive and continuous processes, low numerical and verbal aptitude, and low complexity of functioning with people and data).

A **second study** has examined SES differences among psychotic inpatients admitted to different types of psychiatric facilities (Muntaner et al., submitted). An analysis of covariance was performed, adjusting for gender, length of illness, age and type of psychotic disorder. Results revealed that both **family** and current SES are lower for psychotic patients admitted to state mental hospitals than for psychotic patients admitted to private and university hospitals. A second finding was that for chronic psychotic patients, drift occurred only among psychotic patients admitted to private and university hospitals. Thus, the proportion of psychotic patients experiencing "social drift" after being admitted to a psychiatric facility is limited to those with higher SES.

In a **third study**, structural models of social drift among different DSM-III disorders (Schizophrenia, Bipolar Disorder, Major Depressive Disorder, Schizoaffective Disorder, and other Axis I psychotic disorders) are compared in an effort to characterize differences in patterns of downward social mobility (Muntaner et al., in preparation). The use of structural equation modeling is particularly appropriate to this study, because it allows the investigation of direct and indirect pathways (e.g., an effect of family SES through patient's education) and the simultaneous testing of sociological, psychological and biological variables, and their relationships.

A **fourth** study, in process, examines the social and psychopathological determinants of violent behavior among psychotic patients (Muntaner et al., in preparation). We examine the effects of gender, age, social class (Muntaner, in preparation), social stratification, length of illness, and psychiatric disorders (Schizophrenia, Affective Disorders, Substance Abuse Disorders). The analysis includes bivariate and multiple logistic regression. Because the presence of psychotic symptoms increases the probability of violent behavior (Link et al., 1992), it is important to determine its association with specific psychiatric disorders.

Significance of the research.

The present project contributes to a better characterization of the relationship between SES and mental disorders by means of using modern definitions of what constitutes a psychiatric case and by the sampling of a variety of psychotic disorders. The characterization of the effects of SES on the development and course of psychotic disorders will be a step forward in a discipline such as mental health which is defined at various ontological levels and thus benefits from cross-level fertilization. Because the social epidemiology of infectious and cardiovascular diseases has already shown that social factors determine rates of specific diseases, it is likely that mental disorders are also affected by similar "webs of causation".

Publications

C. Muntaner, A. E. Pulver, W.W. Eaton and John McGrath. "Psychosocial work environment and schizophrenia: the role of low substantive complexity and undesirable working conditions." (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02612-01 LSES

PERIOD COVERED

October 29, 1991 to September 30, 1992

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

Psychosocial Work Environment, Individual Vulnerability and ADMDs Disorder

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

PI: C. Muntaner Visiting Associate LSES, NIMH

COOPERATING UNITS *(if any)*

Department of Mental Hygiene, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS	PROFESSIONAL	OTHER
0.6	0.6	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 present project is an effort to expand the Laboratory's research program to the psychological effects of occupational conditions on the development of specific mental disorders. This project uses the Demand/Control (D/C) model of the psychosocial work environment to investigate the relationship between job conditions, alcohol, drug abuse and mental disorders.

Data from the NIMH/Epidemiological Catchment Area study, a probability sample in five US metropolitan areas, is used as a source of incident cases of specific mental disorders as assessed by the Diagnostic Interview Schedule/DSM-III.

Data analysis includes methods appropriate for nested case-control or case cohort studies such as conditional logistic regression. In addition, confirmatory factor analysis is used to test the dimensional assumptions of the D/C model using a multiple indicators.

The primary aim of the project is to investigate the mechanisms by which the psychosocial structure of work environments contributes to the development of mental disorders.

Project Description

The Laboratory of Socio-environmental studies has developed a substantial body of research on the psychological effects of job conditions. However, the effect of job conditions (substantive complexity, routinization, closeness of supervision, position in occupational structure, job pressures, extrinsic risks and rewards) on modern definitions of psychiatric case (e.g., DSM-III Schizophrenia) has yet to be assessed.

The present project is an extension of the Demand/Control model of the psychosocial work environment (Karasek and Theorell) to the prediction of specific mental disorders. The Demand/Control model of work environment and stress has been developed partly on the basis of the Kohn/Schooler research on the psychological effects of job conditions (Muntaner, in press). Furthermore, the Demand/Control model (D/C) has emerged during the last decade as the dominant model in the area of work and stress, mainly through the empirical support given to the model in the field of cardiovascular epidemiology. The D/C model looks at the context of job conditions in terms of two basic dimensions, control over work and psychological demands. These two dimensions can be used to predict psychopathological consequences of exposure to work environments (Muntaner, in press). The control dimension has two main components: **skill discretion** (learning on the job, creativity, variety of tasks), and **decision authority** (ability to make decisions, ability to influence the group and the organization). The psychological demands dimension refers to how fast and hard the worker has to work, the hectic nature of the job, whether there is enough time to do the job, amount of concentration required, conflicting demands, and frequency of interruptions). These two dimensions describe four kinds of psychosocial work experience generated by varying the levels of psychological demands and decision latitude: high stress jobs (high demands and low control), passive jobs (low demands and low control), active jobs (high demands and high control) and low stress jobs (low demands and high control). In the present project, specific predictions such as "passive jobs will be associated with risk of development of alcoholism" were derived from clinical and experimental studies (e.g. "learned helplessness").

The **first study** in this project was a confirmatory factor analysis of the D/C dimensions in five US metropolitan areas. To test this dimensional structure, we used self-report and observer's rating scales from the Karasek system and from the Department of Labor's Dictionary of Occupational Titles. The results from this study indicated that the D/C model has acceptable goodness of fit indices.

A **second study** is being conducted to determine whether adults working under stressful working psychosocial characteristics might be at risk of drug/dependence syndromes. Prospective analyses using conditional logistic regression were executed with interview data from probability samples of five US metropolitan areas. Cases were defined using case definitions for abuse/dependence syndromes involving controlled substances as assessed by the NIMH/Diagnostic Interview

Schedule. These were administered at baseline and at follow-up one year later. Incident cases were identified by contrasting the diagnostic findings at follow-up with those obtained at baseline. After adjustment for sociodemographic factors and history of alcohol dependence, high levels of physical demands and low levels of control were found to be associated with risk of drug abuse/dependence.

A **third study** has been initiated to replicate the drug abuse findings with alcohol diagnoses in the same cohort study sample.

Other **smaller studies** within this area have involved the validation of instruments for the assessment of propensity to alcohol, drug abuse and mental disorders and the stability of assessment of self-reports of drug use.

Significance of the research

This work will lead researchers to those aspects of the work environment which might place individuals at greater risk of specific mental disorders. Exploring the relation between psychosocial aspects of work and mental disorders by means of a cohort study of a representative sample of the US metropolitan population provides an unique opportunity to increase our knowledge in this area. Past studies on the relationship between work and mental health have often relied on special populations or did not include modern definitions of psychiatric cases. Additional advantages of our methodology include the avoidance of recall bias by using an assessment of work stress which is independent of subjects' perceptions, and the use of small area matching to account for the effect of unmeasured stressors not related to work environments.

In addition, the identification of psychosocial exposures at work can lead to work redesign interventions to diminish the adverse mental health effects of work environment.

Publications

Muntaner, C., Tien, A., Eaton, W.W. and Garrison, R. Occupational characteristics and the occurrence of psychotic disorders. Soc Psychiat Psychiat Epidemiology. 26, 273-280, 1991.

Muntaner, C.. The Demand/Control model of the psychosocial work environment: a behavioral and social critique (in press).

Muntaner, C., Kumor, K., Nagoshi, C. Repeatabilty of cocaine i.v. administration. Brit J Addiction, 87, 591-99, 1992.

Nagoshi, C., Walter, D., Muntaner, C. Validation of the TPQ in a sample of male drug users. Person Indiv Diff, 13, 401-409, 1992.

Cascella, N. & Muntaner, C. Disturbi da cocaina e da sostanze amfetamino simili. In : G.B. Cassano et al. (Eds.) Trattato italiano di Psiquiatria. Milano: Masson (in press).

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

PROJECT NUMBER
Z01 MH 00424-17 LCB

PERIOD COVERED: October 1, 1991 through September 30, 1992

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)

PI's:

Michael J. Brownstein, Chief, Laboratory of Cell Biology, NIMH
T.I. Bonner, Res. Biologist, Laboratory of Cell Biology, NIMH
M. Palkovits, Visiting Sci., Laboratory of Cell Biology, NIMH

COOPERATING UNITS (if any)

Semmelweis University Medical School, Budapest

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 8	PROFESSIONAL: 8	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 structures, distributions, and functions of molecules of importance in the nervous system are being studied. Several cDNAs that encode neurotransmitter receptors have been isolated. In addition, cDNAs encoding a glycine transporter and the vesicular monoamine transporter have been cloned. The distributions of mRNAs to which the above DNAs are complementary have been or are being determined by means of in situ hybridization histochemistry (ISHH) as have the distributions of other mRNAs (e.g., peptide processing proteases, components of second messenger systems). Expression of cloned cDNAs has allowed functional studies to be undertaken.

Other Professional Personnel Engaged on Project

B. Hoffman	Sr. Staff Fellow	LCB, NIMH
T. Usdin	Guest Researcher	LCB, NIMH
E. Mezey	Visiting Scientist	CNB, NINDS
S. Lolait	Guest Researcher	LCB, NIMH
A.-M. O'Carroll	Guest Researcher	LCB, NIMH
G. Harta	Guest Researcher	LCB, NIMH
M. Konig	Biologist	LCB, NIMH
C. Chen	Biologist	LCB, NIMH
B. Borowsky	Guest Researcher	LCB, NIMH
D. Button	Guest Researcher	LCB, NIMH
R. Bhatnagar	Guest Researcher	LCB, NIMH
L. Lautens	Guest Researcher	LCB, NIMH
L. Mahan	Research Biologist	LCB, NIMH

Project Description:

Receptors and transporters

A second vasopressin receptor--the V2 subtype--has been cloned. This receptor's mRNA has only been detected in the kidney to date; it mediates the antidiuretic action of vasopressin. The mRNA encoding the V1a receptor, on the other hand, is in several peripheral organs and in the brain as well.

The gene that encodes the V2 receptor is found on the long arm of the X chromosome. A defect in the coding region of this gene--in the first pedigree studied--is responsible for X-linked nephrogenic diabetes insipidus.

A novel somatostatin receptor, the third described, has been cloned and characterized. This receptor shows marked preference for somatostatin 28 as opposed to somatostatin 14. Several additional orphan receptor clones have been isolated and are being examined.

A second 5HT-1D receptor has been cloned and characterized. This is the target of a novel class of drugs used to treat migraine.

A second glycine transporter cDNA has been isolated. The mRNA encoding this transporter seems to arise from the same gene as the mRNA encoding the glycine transporter described by P. Hartig and his coworkers. The two transporters have different N-terminal sequences and different tissue distributions. Inhibitors of these transporters may be useful for treating spasticity and may be useful as sedatives or anticonvulsants.

The CNS vesicular monoamine transporter has been cloned. This transporter appears to span the vesicular membrane 12 times. Unlike the monoamine uptake proteins, which are sodium cotransporters, it is a proton antiporter and is inhibited by reserpine and tetrabenazine.

A method has been developed for identifying agonists or antagonists acting on cloned receptors that increase intracellular calcium.

The cellular localizations of histamine, dopamine, and acetylcholine receptors in the stomach and duodenum have been examined. Unexpectedly, these receptors are all found on immunocytes, not epithelial cells.

Significance to Biomedical Research

Nerve cells use chemical "transmitters" to communicate with one another and with 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 16 years the number of putative neurotransmitters has increased by a factor of four of 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 and their receptors remain to be isolated and characterized. The work outlined above is principally devoted to improving our understanding of neurons. 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 continue.

Publications

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Demchyshyn L, Sunahara RK, Miller K, Teitler M, Hoffman BJ, Kennedy JL, Seeman P, Van Tol HHM, Niznik HB. A human serotonin 1D receptor variant (5HT1db) encoded by an intronless gene on chromosome 6, Proc Natl Acad Sci USA 1992;89:5522-6.

Erickson JD, Eiden LE, Hoffman BJ. Expression cloning of a reserpine-sensitive monoamine transporter, Proc Natl Acad Sci USA 1992;in press.

Grewal RP, Martinez M, Hoehe M, Bonner TI, Gershon ES, Detera-Wadleigh S. Genetic linkage mapping of the m4 human muscarinic receptor (CHRM4), Genomics 1992;in press.

Hoffman BJ, Mezey E, Brownstein MJ. Cloning of a serotonin transporter affected by antidepressants, Science 1991;254:579-80.

Kenakin TP, Bond RA, Bonner TI. Definition of pharmacological receptors, Pharmacol Rev 1992;in press.

Konig M, Mahan LC, Marsh JW, Fink JS, Brownstein MJ. Method for identifying ligands which bind to cloned G_s- or G_i-coupled receptors, Mol Cell Neurosci 1991;2:331-7.

Leonhardt S, Weisberg E, Hoffman BJ, Tietler M. Molecular pharmacology differences in the interaction of serotonin with 5HT1C and 5HT2 receptors, Mol Pharmacol 1992;in press.

Lolait SJ, O'Carroll AM, McBride OW, Konig M, Morel A, Brownstein MJ. Cloning and characterization of a vasopressin V2 receptor and

possible link to nephrogenic diabetes insipidus, Nature 1992;357:336-9.

Matsuda LA, Lolait SJ, Brownstein MJ, Bonner TI. The THC receptor and its implications. In: Proceedings of Conference on Biological Basis of Addiction, Oxford University Press;in press.

Mezey E, Kiss JZ. Coexpression of vasopressin and oxytocin in hypothalamic supraoptic neurons of lactating rats. Endocrinology 1991;129:1814-20.

Mezey E, Leranth C, Eskay R, Horvath S, Palkovits M. The origin of somatostatin-containing nerve fibers innervating the hypothalamic supraoptic nucleus. Brain Res 1991;554:293-8.

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Morel A, O'Carroll AM, Brownstein MJ, Lolait SJ. Molecular cloning and expression of a rat V1a arginine vasopressin receptor, Nature 1992;356:523-526.

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Porteus MH, Brice AEJ, Bulfone A, Usdin TB, Ciaranello RDC, Rubenstein JLR. Isolation and characterization of a library of cDNA clones that are preferentially expressed in the embryonic telencephalon, Mol Brain Res 1991;12:7-22.

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Usdin TB, Brownstein MJ, Moss B, Isaacs SN. A recombinant vaccinia virus encoding bacteriophage SP6 RNA polymerase allows

rapid, efficient expression of cloned genes in tissue culture, Biotechniques 1992;in press.

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

PROJECT NUMBER
Z01 MH 02593-02 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Modulation of Protein and Cell Membrane Function by Heparin/Heparan Sulfate

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

PI:	A. L. Stone	Sr. Investigator	LCB, NIMH
Others:	G. E. Deibler	Research Chemist	LCM, NIMH
	S. V. Spitsin	Fogarty Fellow	LCB, NIMH

COOPERATING UNITS (if any)
Clin. Pharm. Br., Lab. Tumor Cell Biology and Frederick Cancer Res. and Develop. Ctr., Developmental Therapeutics Prog, NCI; Lab. of Developmental and Molecular Immunity, NICHD

LAB/BRANCH
Laboratory of Cell Biology

SECTION

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.13	1.5	0.63

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.)

Heparin/heparan sulfate (H/HS)- like oligosaccharides (S-OligoS) were studied in vitro to elucidate their multifunctional modulation of protein and cell membrane function: 1) Struc/fxn of heparin substitute, SP54, which like H/HS inhibits infectiousness of HIV-1 in vitro, was continued. Capacities of 17 newly purified MW components (Cpts) of SP54 to inhibit HIV-1-induced syncytia formation and cytotoxicity confirmed [ref. Z01 MH 02593-01] that Cpts of > 5000 MWapp and a specific ~octaS (~1.8% of SP54) both were highly active inhibitors of cytotoxicity [EC50=200 ng/ml and 580 ng/ml], while high potency inhibition of syncytium formation resided solely in Cpts > 5000 MWapp [EC50 = 200-300 ng/ml]. This first demonstration of structural specificity and different structural requirements for the inhibition of HIV-1 "cell entry" and cell fusion by S-oligoS offers potential adjunct to AIDS therapy and understanding of viral cell reactions. 2) In vitro inhibition by sulfated ß- cyclodextrin of growth of Kaposi Sarcoma cell lines derived from AIDS patients [EC50 ~15 ug/ml] also exhibited differential activity: 7 sugars and an av of 1S/sugar was required, with highest potency (5-10-fold enriched) in Cpt(s) having less than max av S. 3) Modulation of basic fibroblast growth factor (bFGF) by H and suramin (S) was demonstrated by CD and fluorescence spectroscopy to involve direct conformational alteration of its receptor-binding region. S was bound within 1 nm of the sole bFGF tryptophan.

Individual Project Description:

This project studies struc/fxn of heparin and heparin-like class of sulfated glycosaminoglycans (H/HS, S-oligoS), which are components of cells and the extracellular matrix, including that of brain (ref. to Z01-MH 0136- 5-12 LNB). They modulate biological activities of proteins and cell membranes in numerous normal functions, and in current and developing treatments of disease: e. g., modulation of anticoagulation and other enzymes, development/cell-cell recognition including myelination, San Filippo mental retardation, stimulation/ inhibition of normal and tumor cell growth/ angiogenic potential, virus-host cell receptor reaction, and lymphocyte immunogenic and homing reactions; as low affinity receptors for protein growth factors/induce high affinity GF-cell receptor binding. Except for Rosenberg's seminal studies that elucidated the molecular basis of mutiple actions of H/HS in anticoagulation, struc/fxn of H/HS remains largely undetermined. With Rosenberg, we showed that multiple conformational changes induced in antithrombin by his specific anticoagulant H was a basis for the multiple modulation of antithrombin's neutralization of the clotting enzymes. We also proposed a sequence for this multifunctional eicoS, suggesting its general significance as a structural element for other systems. Understanding structural specificity of the numerous H/HS functions would enhance their usefulness.

Last year we initiated studies of struc/fxn of S-oligoS *in vitro* inhibition of infectivity of HIV-1, and in two other systems, *in vitro* model for inhibition of angiogenic potential of Kaposi sarcoma (KS) (collab'n/Dr. Browning), and modulation of basic fibroblast growth factor (BFGF) by H/HS and suramin (collab'n./Dr. Ranson). These 3 studies were continued and extended this year; Dr. Sergey V. Spitsin joined us mid-year in study of putative S-oligoS-T4 cell (ligand-receptor) reactions. We completed the study on a structural basis for immunologic and antigenic activity of Vi antigen, a new typhoid vaccine and remain in collab'n/Drs. Robbins, Szu. Our study with Deibler et al, struc/fxn of human myelin basic protein (MBP) Cpts, now extends to comparison of their immunological function.

1). Dr. Audrey L. Stone, Sr. Scientist , LCB, NIMH-IRP; FCRDC

TO: study struc/fxn of the heparin substitute, SP54 (a mixture of disulfated xylose (DiSX)-containing oligoS), known, as are H/HS and other sulfated glycans, to inhibit infectiousness of HIV-1 *in vitro*:

MW Cpts were prepared BY LP chromatography on Biogel P10 and quantitative spectroscopy/ analyses; bioassayed: twice in duplicate for inhibition of *in vitro* killing of T4 lymphocytes by HIV-1 BY the soluble formazan reaction for viable cells (FCRDC); syncytia formation (BYfusion between gp120-bearing and uninfected CD4-bearing cells, measured at 48 h); and data expressed BY dose-response curves. Molecular modeling was BY building SP54 octaS with C-K space-filling atoms. Last year we showed that Sp54 contained Cpts of ~ 30 to 1 Kda in MWapp and that a sparsely sulfated Cpt, having low anti-HIV-1 potency [and BY mixed assay did not inhibit active Cpts] and low MW (around 2Kda), comprised~45 % of recovered SP54. Potency of Cpts vs HIV-1 cytotoxicity (measured 7 d after *in vitro* infection) showed a biphasic relation with MWapp: high potency resided in the high MW Cpts, probably in segments of ~5 - 7 Kda MWapp, and in one lower MWapp Cpt, ~octaS; This size/function resembled that of Rosenberg's multifunctional H. Thus, HIV-1 infectiousness might involve H/HS structures, and inhibition of virus might be mediated by H-like structures contained more-or-less in the various inhibitory sulfated glycans [Our models of S-oligoS indicated that the alpha-glucuronic acid residues present in SP54, could twist back to-wards or cause bends in the chain, enabling a limited "helical" arrangement of sulfated and unsulfated hydroxyls]. Study of inhibition HIV-1-induced syncytia by these Cpts might provide further insight.

TO this end: this year, 17 of 20 newly purified Cpts were assayed for inhibition syncytia and cytotoxicity. Data confirmed that Cpts > 5000 MWapp, and an octa-hexaS-Cpt(s) comprising 1.8% of the wt, expressed high potency [EC50=200 and 580 ng/ml, resp.] while Cpts < 5000 MWapp showed EC50 increasing 2 - 70 ug/ml. Syncytia-inhibiting activity, however, apparently resided solely in the relatively high MWapp Cpts >5000 [EC50=200-300 ng/ml]. EC50 increased to 2 -10 ug/ml forMWapp <5000.

Our data were the first to show differential anti-HIV-1 activity *in vitro* by SP54 Cpts. Now we reveal their differential structural requirements for inhibition of "cell entry" and cell fusion. Significance: 1) findings are consistent with our idea on geometries of S-oligoS functional units, and elucidate possible reactions involved in syncytia formation 2) provide a rational struc/fxn for SP54 and similar S-oligoS in the inhibition of HIV-1 infectivity 3) offer promise of an inexpensive adjunt to AIDS therapy and amelioration of behavioral effects. Purification of and struc/fxn studies of active Cpts will continue; a new method for assaying size of syncytia will be employed. Anticoagulation assays will be performed.

2) Dr. Audrey L. Stone, Dr. Philip Browning, Scientist, LTCB, NCI :

TO: investigate sulfated cyclodextrins(antiangiogenics) as potential agents for inhibition of AIDS-related KS: Cpts of S-bCD (heptamer) were purified BY chromatography, metachromatic analyses and characterized by degree of sulfation and anionic density. S-bCD and Cpts, and S-aCD (hexamer) were tested for inhibition of angiogenic-dependent cell growth BY *in vitro* bioassays against growth of several

cell lines derived from AIDS patients, as measured BY ^{3}H-thymidine-uptake (at 4 d) and count of trypsin-dissociated cells (at 7 d).

We had shown S-bCD to be inhibitory against a KS cell line and potencies to differ among Cpts (S-aCD and unsulfated CDs were inactive). This year, S-bCD and newly prepared Cpts also exhibited potency against newly established, more sensitive, KS cell lines [EC50 S-bCD=15 ug/ml]. Cpts differed in potency. Those having < 1 av sulfate per sugar were not active up to 200 ug/ml. The most active Cpt, however, did not have the highest degree of sulfation, indicating a specificity of structure inthis function. Further Cpts have been purified for bioassay.

Control of growth of KS in AIDS patients, without concomitant toxic effects, requires new approaches and drugs. The significance of these data on struc/fxn S-bCDs is their suggestion of specificity and a new avenue to therapy having less toxicity and cost. Studies will continue.

3) Dr. Audrey L. Stone, Dr. Malcom Ranson, Visiting Fellow, Clinical Pharmacology Branch, NCI

TO study the molecular basis of inhibition of bFGF by suramin (S), (a new anti-cancer, growth factor antagonist) and modulation of bFGF function by H/HS (low affinity bFGF receptor), BY low uv circular dichroism (CD) (and conformational analysis, CA) and fluorescence (Flu) spectroscopy (static, global lifetimes, anisotrophy) using human recombinant bFGF: Binding of bFGF to a H/HS-like receptor on target cell converts bFGF from a medium to a high-affinity ligand for its signal-transducing cell receptor (CR). One H-binding site is adjacent to the CR-binding site (CR-BS) of bFGF, which includes the sole W of the protein. We asked whether H and /or S evoked its modulation by direct conformational effect (s) on the CR-BS. We are the first to demonstrate that both H and S directly perturb the conformations involving W, thereby reflecting structural alteration(s) in CR-BS that are also reflected in changes in low uv CD.

1) S binds close to the CR-BS of bFGF, within 0.99nm of W as demonstrated from data on complete quenching of static Flu, stoichiometry ~1, providing a molcular basis for its inhibitory action and a screening method for drug development; 2) H induced 2 conformational changes involving CR-BS, manifest at low and higher ratios to bFGF, as demonstrated by: a) 2 phases of static Flu enhancement by H, the second accompanied by decrease in Flu of tryosines(s); b) lengthening of lifetimes of decay-associated spectra by Global Analysis, at low ratios of H, with a reducton of this effect at higher ratios; c) bFGF fluorochrome exhibits fast (~5%) and slower (~95%) rotational correlation coefficients that are affected differently at low and higher ratios of H,indicating 2 kinds of changes in the environmental interactions perturbing rotational freedom of W, and in aggregation of bFGF (slow decay associated with rotation of the protein). 3) CD/CA of bFGF showed relative absence of alpha helix and a large proportion of beta-structure in solution, consisent with concurrently published X-ray data of a beta-barrel structure. H induced changes in the low uv CD, which again reflected 2 phases of change, appeared by CA to stabilize a short alpha helix and/or a beta-turn; S appeared to stabilize beta-structure.

These results are seminal to the elucidation of molecular interactions that subserve modulation of bFGF function by H/HS and S. Studies will continue, incl. near UV CD, and usefulness explored on a similar approach on H/HS modulation of PDGF, a factor highly important in neuroglycobiology.

4) Dr. Audrey L. Stone, Gladys Deibler, Research Chemist, LCM, NIMH-IRP

TO: study struc/fxn of human CNS MBP (expt'l autoimmune encephalitogenic antigen) BY conformational studies on 3 MBP Cpts using CD spectroscopy, CA, and *in vitro* assays of immunological function (R. Voskuhl, NINDS) BY using standard chromium release assay for cytotoxicity evoked by MBP-specific T8 cell lines (effector cells) against autologous B-cell lines (target cells), in the presence of Cp 1 (major 18.5 Kda MBP Cpt), CpP_{T96} (monophosphorylated form at the single threonine) and Cp 17.2 (exon 5 deleted). CD/CA indicated that CpP_{T96} and Cp 17.2 both had increased potential for beta-structure folding . Assays of Cp 1 and CpP_{T96} , however, showed no difference in ability to elicit cytotoxic reactions compared with control antigen. Studies are continuing using other cell lines.

Honor: Invitation by Ed., to contribute a paper to a Thematic Issue of Carbohydr. Res. on Proteoglycans.

Publications:

1) Stone AL Spitzin SV Melton DJ Structure-function relations of heparin substitute, SP54, in inhibition of human immunodeficiency virus (HIV-1) cytotoxicity and syncytium formation *in vitro*. An. Mg. Soc. Complex Carbohydrates abs., Nashville, 1992; in press. 2) Deibler GE Burlin TV Stone AL Purification and structural studies on human myelin basic protein. Trans. Am. Soc. Neurochem. abs 202 1992; 191 3)Szu SC Li Xr Stone AL Robbins JB. Relation between the structure and some immunologic properties of the Vi, capsular polysaccharide, Infection and Immunity, 1991;59:4555. (published after last report)

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

PROJECT NUMBER
Z01 MH 00422-21 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

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:	Martin Zatz	Section Chief	SBP, LCB, NIMH
Others:	Nancy Lee	IRTA Fellow	SBP, LCB, NIMH
	M. Wolfe	PRAT Fellow	NIGMS
	M. Rollag	Visiting Scientist	USUHS, Bethesda

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 STAFF YEARS: 4.8	PROFESSIONAL: 2.8	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.)

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. Cyclic AMP is a key regulator of melatonin production by these cells. Light and L-type calcium channels act in part through cyclic AMP but the circadian pacemaker does not. Regulation of melatonin production occurs via induction of the enzyme serotonin N-acetyltransferase. Lability of this enzyme has prevented purification or gene identification, but progress has been made toward increasing its amount and stability.

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, cell culture, and radioactive trace techniques.

Major Findings: We have developed a system using dispersed chick pineal cells in static culture, which displays a circadian rhythm of melatonin release for at least two weeks under cyclic lighting conditions, and for at least four 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.

Cyclic AMP is a key regulator of melatonin production in the chick pineal gland. Agents that raise cyclic AMP levels (such as forskolin), or cyclic AMP analogs (such as 8-bromocyclic AMP), increase melatonin synthesis and release, whereas agents that lower cyclic AMP levels (including light) decrease melatonin synthesis and release. The circadian oscillator in these cells also raises and lowers melatonin output. We have been investigating the relationships between cyclic AMP and the circadian pacemaker in the regulation of melatonin production. In chick pineal (unlike certain neuronal systems) the weight of the evidence indicates that cyclic AMP is not on an entrainment pathway to the circadian pacemaker. Instead, cyclic AMP appears to act downstream from the pacemaker. The pacemaker might itself act directly

through cyclic AMP, regulating melatonin content by raising and lowering cyclic AMP levels. If this were the case, and if the effects of cyclic AMP levels on melatonin output are saturable (as they must be), then, in the face of such saturating levels of cyclic AMP, the pacemaker should no longer raise or lower melatonin output. To test this prediction, maximally effective concentrations of forskolin and 8-bromocyclic AMP were determined. Both agents markedly increased melatonin output. After 36 hours, cells were refractory to additional stimulation of melatonin output by addition of both agents together, or by higher concentrations of forskolin (although cyclic AMP levels could still be raised further). Nonetheless, the circadian pacemaker continued to raise and lower melatonin output--the rhythm persisted in the face of saturating levels of cyclic AMP. It is suggested, therefore, that the circadian pacemaker in chick pineal cells acts with, not through, cyclic AMP to regulate melatonin synthesis. Cyclic AMP and the pacemaker act synergistically to regulate serotonin N-acetyltransferase activity and the melatonin rhythm, with cyclic AMP mediating acute effects and amplitude regulation.

Another group of agents that acutely change melatonin synthesis and release are those that affect calcium influx through L-type voltage-sensitive calcium channels (VSCC). These channels on chick pineal cells were demonstrated and characterized electrophysiologically. Agents which promote calcium influx through these channels, such as Bay K 8644, acutely increase melatonin output and agents which retard calcium influx such as nitrendipine, cobalt ion, and low external calcium concentrations, acutely decrease melatonin output. Our previous experiments examined the interactions among the effects of cyclic AMP and calcium-related agents on melatonin output and suggested that changes in calcium influx might act *through* cyclic AMP to affect melatonin production. Effects of calcium-related agents and manipulations on cyclic AMP (and cyclic GMP) levels have now been demonstrated directly. These effects support a role for cyclic AMP (but not cyclic GMP) in the effects of changes in calcium influx

on melatonin production by these cells.

Serotonin N-acetyltransferase (NAT), the rate limiting enzyme in melatonin production in the pineal gland, has eluded purification due to its short half life and instability in homogenates. The chelator EGTA protected NAT activity in chick pineal homogenates. Results indicated that EGTA is unlikely to protect NAT by removal of a harmful metal. Cold storage of homogenates temporarily increased activity and addition of certain proteins (BSA, human albumin, trypsin soybean inhibitor) protected NAT activity against heat inactivation. Other proteins (ovalbumin, myoglobin, hemoglobin) and various protease inhibitors (aprotinin, leupeptin, PMSF, pepstatin, E64), however, neither protected nor affected NAT activity. High phosphate levels increased NAT activity 3-5 fold, compared to 50 mM phosphate, yet had no protective effect. Various nucleotides, the phosphatase inhibitor okadaic acid and the catalytic subunit of protein kinase A failed to mimic or inhibit phosphate-induced increases in NAT activity. Neither the addition of nondenaturing detergents (Triton-X and CHAPS) nor centrifugation significantly affected NAT activity in the presence of high or low phosphate. Determination of conditions and mechanisms involved in the stabilization of NAT would permit its purification and, subsequently, elucidation of its molecular and gene regulation.

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: Pharmacologic agents acting on the pacemaker will be sought; the effects of temperature on melatonin production, pacemaker function, photic responses

and gene regulation will be explored; identification and dynamics of transcription factors involved in circadian and photic regulation, and melatonin production, will be sought; means to stabilize and purify NAT and/or amplify its gene expression, and identify its gene, will be explored.

Publications:

Zatz M. Light and norepinephrine similarly prevent damping of the melatonin rhythm in cultured chick pineal cells: Regulation of coupling between the pacemaker and overt rhythms?, J Biol Rhythms 1991;6:137-47.

Zatz M. Neuropharmacology of the SCN; Introduction. In: Klein DC, Moore RY, Reppert SM, eds. The suprachiasmatic nucleus: the mind's clock. New York: Oxford Press, 1991;260-2.

Zatz M, Wang HM. Low salt mimics the effects of dark pulses on the circadian pacemaker in cultured chick pineal cells, Am J Physiol 1991;261:R1424-30.

Zatz M. Perturbing the pacemaker in the chick pineal, Discussions in Neurosci 1992;8:67-72.

Zatz M. General and section introductions. In: Zatz M, ed. Circadian rhythms. Discussions in Neurosci 1992;8.

Zatz M. Agents that affect calcium influx can change cyclic nucleotide levels in cultured chick pineal cells, Brain Res 1992;in press.

Zatz M. Does the circadian pacemaker act through cyclic AMP to drive the melatonin rhythm in chick pineal cells?, J Biol Rhythms 1992;in press.

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

PROJECT NUMBER
Z01 MH 00434-11 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

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
	Christian Felder	Sr. Staff Fellow	LCB, NIMH
Others:	Alice Ma	Biologist	LCB, NIMH
	Eileen Briley	Biologist	LCB, NIMH
	Lianne Bently	Student	Marymount Univ.
	Christina Jose	Student	Georgetown Univ
	Mike Levine	Student	HHMI Summer Student
	Laura Lautens	Guest Researcher	LCB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Unit on Pharmacology

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NIMH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6	2	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.)

Studies of muscarinic m1, m3, or m5 receptor-mediated signal transduction demonstrated simultaneous stimulation of phospholipase A2, phospholipase C, Phospholipase D, adenylate cyclase, tyrosine kinase, and calcium influx. Calcium influx activation and tyrosine phosphorylation occurs transduction pathways. Muscarinic receptor-induced tyrosine phosphorylation was calcium influx dependent and resulted in the tyrosine phosphorylation of phospholipase-C-gamma.

Muscarinic m1, m3,, and m5 receptors induce a morphology change and have a tumor suppressor function when expressed in CHO cells. The morphology change was independent of all signal transduction pathways tested except receptor-operated calcium influx and tyrosine phosphorylation. Tyrosine phosphorylation was calcium influx dependent. Muscarinic receptors-operated calcium channel activation plays a central role in CHO cell tumorigenicity.

Human and rat cannabinoid receptors expressed in CHO or L cells were linked to the inhibition of adenylate cyclase. Other Gi - coupled receptors linked to the inhibition of adenylate cyclase, such as alpha2, D2, m2, and m4 receptors were shown to augment ATP-stimulated phospholipase A2, but not the cannabinoid receptor. Cannabinoid receptor-activation had no effect on phospholipase A2, or C, although, high concentrations of cannabinoid agonists stimulated the release of arachidonic acid, intracellular calcium, and the inhibition of arachidonic acid uptake in a receptor-independent manner.

Project Descriptions

Muscarinic receptor-operated calcium channel activation.

Christian Felder previously showed muscarinic receptor activation of calcium influx through a voltage independent calcium channel in CHO cells that was independent of inositolphosphate release. The calcium channel was not blocked by voltage-operated calcium channel (VOCC) blockers and was not activated with BAY-K, an activator of VOCCs. Calcium influx was blocked with heavy metals, further suggesting it was a calcium channel. Attempts at whole cell recordings to analyze the channel through electrophysiological techniques, in collaboration with Mike Poulter of NINDS, were unsuccessful due to the channel's low conductance, low abundance, or both. Previous studies by Christian Felder identified CAI, a blocker of muscarinic receptor-operated calcium channels, that had little effect on other signal transduction pathways. Other compounds have been identified with similar activity including econozole, miconozole, and two Chinese herbal medicine extracts. Some of these compounds may prove useful as ligands for binding studies or affinity column purification of the channel. Using chimeric muscarinic m2 and m3 receptors in which the third cytoplasmic loop and putative G protein binding domain had been exchanged, it was concluded that a portion of the m3 receptor other than the third cytoplasmic loop was responsible for coupling to the calcium channel. Attempts to determine the structural requirements for calcium influx regulation are ongoing.

Tumor suppressor function of muscarinic acetylcholine receptors in CHO cells is the result of receptor-operated calcium influx.

Christian Felder in collaboration with Elise Kohn of the NCI, Fabian Gusovsky of NIDDK, Linda MacArthur and Alice Ma of LCB-NIMH demonstrated a m5 muscarinic receptor-induced morphology change when the receptor was expressed in CHO

cells. The morphology change was agonist dependent and blocked with a muscarinic receptor antagonist. The muscarinic receptor agonist, carbachol, induced the suppression of colony formation in soft agar and tumors in nude mice which were also blocked with the addition of muscarinic receptor antagonist. Of the 5 signal transduction pathways examined which were activated by m5 receptors, only calcium influx correlated with the antagonist-induced morphology change. Previous studies in this lab indicated that m5 receptors were coupled to the opening of receptor-operated calcium channels that provides the cell with a sustained increase in intracellular calcium. Our studies indicate that the sustained incresae in intracellular calicum is at least in part responsible for m5 recptor-induced tumor suppression.

m1, m3, m5 muscarinic receptors stimulate tyrosine phosphorylation of phospholipase C-gamma through activation of receptor-operated calcium channels.

Fabian Gusovsky of NIDDK and Christian Felder has shown a m1, m3, and m5 muscarinic receptor dependent tyrosine phosphorylation of phospholipase C-gamma. This subtype of phospholipase C is normally associated with the growth factor family of receptors which have an endogenous tyrosine kinase activity within their structure. Carbachol stimulated tyrosine phosphorylation over a concentration range that correlated with muscarinic receptor-operated calcium influx and not with muscarinic receptor-stimulated phospholipase A_2, C, D, or adenylate cyclase activation. Tyrosine phosphorylation was blocked with removal of extracellular calcium and with the addition of the receptor-operated calcium channel blocker CAI. Carbachol-stimulated inositolphosphate release was reduced 30% with the removal of extracellular calcium, the addition of CAI, or the addition of a tyrosine kinase inhibitor. These data indicate that m1, m3, or m5 receptors can stimulate both G protein coupled phospholipase C-beta and tyrosine kinase regulated phospholipase C-gamma.

Signal transduction associated with the vasopressin V1a receptor.

Eileen Briley in collaboration with Christian Felder and Julius Axelrod has investigated the signal transduction pathways associated with the vasopressin V1a receptor cloned by Stephen Lolait. The V1a receptor stimulated the release of inositol phosphates as previously demonstrated by other laboratories. It was further discovered that the V1a receptor can stimulate calcium influx, phospholipase A_2 and phospholipase D, but had no effect on cAMP accumulation. Both phospholipases A_2 and D were dependent on calcium influx. Therefore, V1a receptors can couple to multiple signal transduction pathways simultaneously.

Expression of cloned $cPLA_2$.

Recently a high molecular weight cytosolic phospholipase A_2 ($cPLA_2$) was purified and cloned by investigators at Eli Lilly Co. In collaboration with Ruth Kramer and John Sharp of Eli Lilly Co., Christian Felder, Laura Lautens and Julie Axelrod are currently attempting to stably express $cPLA_2$ in CHO cells already expressing the m5 receptor to determine if this enzyme is activated following m5 receptor stimulation.

Significance to Biomedical Research.

Drugs used in the treatment of mental illness, particularly depression and schizophrenia, and many drugs of abuse function by interacting with membrane neurotransmitter receptors. To fully understand the effect of neurotransmitters on cell function requires detailed knowledge of signal transduction systems. Transmembrane signally pathways begins with ligand binding to a specific cell surface receptor. Activated receptor proteins, G proteins, and effector enzymes sequentially generate a cytoplasmic second messenger which mediates the biological consequence of the ligand in question. Expressing cloned receptor, G protein, or effector DNA into mammalian

transformed cell lines simplifies the study of transduction pathways, and this knowledge can be applied to both neuronal and non-neuronal cells in primary culture.

Proposed Course of Project.

Our laboratory will focus on two signal transduction pathways over the next year. Little is know about receptor-operated calcium channels which we have identified coupled to m1, m3 m5, ATP, and V1a receptors in CHO cells. In collaboration with Hemin Chin of NINDS we will attempt to clone this channel using expression or homology cloning. We will continue our search for drugs that either stimulate or inhibit the channel similar to the actions of maitotoxin and CAI respectively. Some of these compounds may be useful for radioligand binding studies or affinity purification of the calcium channel. Receptor-stimulated calcium influx plays a central role in the regulation of cell growth and proliferation. We have identified a regulatory pathway involving tryosine kinase activation and phosphorylation of phospholipase C-gamma regulated by muscarinic receptor-mediated calcium influx. Second, we will continue to study the signal transduction pathway associated with phospholipase A_2 activation or inhibition. Arachidonic acid and its eicosanoid metabolites have been shown to play an important role in the regulation of action potentials in neural cells, long term potentiation, inflammation, stroke, as well as cell growth and proliferation. The recently cloned $cPLA_2$ appears to be the long sought after effector of receptor-mediated arachidonic acid release. We will express this clone stably in clonal cells lines along with receptors known to stimulate arachidonic acid release to study its regulation in detail. The relationship between receptor-operated calcium channels and phospholipase A_2 regulation will be investigated.

Publications

Felder CC, Ma AL, Liotta L, Kohn EC. The antiproliferative and antimetastatic agent L651582 inhibits calcium influx and arachidonic acid release, J Pharmacol Exp Therap 1991;257:967-71.

Felder CC, Williams H, Axelrod J. A Novel transduction pathway associated with G_i coupled receptors that amplifies ATP-mediated archidonic acid release, Proc Natl Acad Sci USA 1991;88:6477-80.

Felder CC, Poulter M, Weiss, J. Receptor-oprated Ca^2 influx, independent of inositol phosphates and intracellular calcium release, Proc Natl Acad Sci USA 1991;89:509-13.

Uhlen S, Xia Y, Chhajlani V, Felder CC, Wikberg JES. [^{3}H]-MK 912 binding delineates two alpha-adrenoceptor subtypes in rat CNS one of which is identical with the cloned pA2D $alpha_2$-adrenoceptor, Br J Pharmacol, In Press.

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

PROJECT NUMBER
Z01 MH 02386-06 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

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	Chief, UMCN	LCB, NIMH
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	Anna Iacangelo	Microbiologist	LCB, NIMH
	Linda MacArthur	IRTA Fellow	LCB, NIMH
	Dianne Rausch	Staff Fellow	LCB, NIMH
	Yousef Anouar	Visiting Fellowing	LCB, NIMH
	Jeffrey Erickson	Guest Researcher	LCB, NIMH

COOPERATING UNITS (if any)

R. Eskay	Chief, Neurochem.	NIAAA
R. Fischer-Colbrie	U of Innsbruck, Austria	
E. Weihe	U of Mainz, FRG	

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Unit on Molecular and Cellular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Bethsda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7	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.)

We are studying the structural and genetic basis for generating the diversity of neuropeptide/neurotransmitter phenotypes found in the nervous and endocrine systems. This work involves i) analysis of cis-acting elements determining cell-specific expression and regulation of neuropeptide genes and ii) characterization of the components of neuropeptide/neurotransmitter vesicles responsible for packaging of neurotransmitters and neuropeptides in neuroendocrine cells.

1) Positive regulation of enkephalin expression by protein kinase A and calcium, and negative regulation by protein kinase C occurs directly via altered transcription of the enkephalin gene in chromaffin cells. The bovine enkephalin gene is identical to the rat and human genes (for which positive regulation by PMA has been noted). Thus PMA response element can confer both positive and negative regulation as a function of cell type.

2) Primary cultures of bovine chromaffin cells are used to study cell-specific expression of enkephalin, galanin and chromogranin A, via transfection of promoter/reporter gene constructions. Each of these genes is specifically expressed in bovine chromaffin cells, but each has a unique mode of cell-specific regulation.

3) Neuropeptide and secretory protein (chromgranin) biosynthesis are independently regulated in chromaffin cells: biosynthesis of the latter is constitutive rather than regulated in consistent with a role of in constitutive vesicular biogenesis. Studies of the catalysis of secretory vesicle morphogenesis by expression of a chromogranin A expression plasmid in non-endocrine, non-vesicle-containing recipient cells is underway.

4) A cDNA encoding the monamine vesicular transporter (MAT) has been cloned, sequenced and expressed in fibroblastic cells containing a vacuolar-type ATPase.

Project Description:

Neurons and endocrine cells express a very great diversity of specific neuropeptides and neurotransmitters, and package them within secretory vesicles. The types of messenger molecules within the secretory vesicles, together with the rate and pattern of secretion of these messenger molecules upon stimulation of cells at their specific locations in the nervous and endocrine systems, represent the signalling code for intercellular communication in the diffuse neuroendocrine network.

We are attempting to study the structural basis for expression and regulation of the enkephalin, galanin and chromogranin A genes in primary neuroendocrine cells of the adrenal medulla. Dr. MacArthur has demonstrated that increased expression of the enkephalin gene in bovine chromaffin cells upon cell depolarization and activation of the protein kinase A pathway is mediated by an increase in transcription from the enkephalin gene. Increased expression following cell depolarization or protein kinase A stimulation is blocked by treatment with phorbol ester, which also blocks the increase in enkephalin gene transcription induced by depolarization and increased intracellular cyclic AMP. The enkephalin gene in the adrenal medulla contains two specific DNase hypersensitivity sites not found in the gene when examined in other bovine tissues not expressing enkephalin. These sites represent presumptive regions of binding of protein factors that subserve enkephalin gene expression in the adrenal medulla. Enkephalin genomic DNA harvested from chromaffin cells stimulated with potassium is relatively more sensitive to DNase digestion than enkephalin genomic DNA harvested from unstimulated cells. Together with previous data demonstrating that the enkephalin gene is silenced by blockade of electrical activity in developing rodent spinal cord neuronal cells in primary culture (Agoston et al, 1990), these data suggest that enkephalin expression is driven by neuronal activity via intracellular calcium, at the level of the enkephalin gene promoter. These observations form an experimental basis for identifying protein factors in primary

neuroendocrine cells that interact with one or more sites on the enkephalin gene and promote gene transcription, and whose activity is positively regulated by calcium, and negatively regulated by protein kinase C signalling pathways. In contrast, galanin gene expression is strongly positively regulated by activation of protein kinase C following phorbol ester treatment in chromaffin cells. Dr. Anouar has demonstrated that less than 2 kilobases of the galanin promoter fused to a luciferase reporter gene is sufficient to confer robust expression in chromaffin cells but not primary non-chromaffin cells derived from the bovine adrenal gland. The chromogranin A gene appears to represent yet a third mode of endocrine cell-specific regulation. Anna Iacangelo has demonstrated that promoter/reporter constructs comprising less than 600 bases of the chromogranin A promoter exhibit equally strong expression in both chromaffin and non-chromaffin cells, while those including more than 2 kb of 5' flanking DNA are weakly expressed in non-chromaffin cells, but retain expression in chromaffin cells. These results suggest that the cell-specific expression of the chromogranin A gene occurs via a repressor element in the 5' distal portion of the promoter that is active in non-chromaffin cells, but inactive in chromaffin cells.

The amine neurotransmitter content of secretory vesicles is also a critical component of the signalling output of neuroendocrine cells. It is determined both by the expression of enzymes for the biosynthesis of biogenic amine and amino acid transmitters, and the expression of specific transport molecules on the membrane of the vesicle governing its ability to concentrate neurotransmitter molecules within the vesicle for quantal release and signalling to neighboring cells. Jeffrey Erickson and Beth Hoffman (LCB), have cloned and sequenced the rat CNS biogenic amine (norepinephrine, dopamine and serotonin) vesicular transporter. Mr. Erickson has expressed the MAT clone in a fibroblast cell line and reconstituted transport and accumulation of biogenic amines in an intracellular acidic compartment. The demonstration that biogenic amine transport requires only the specific transporter molecule and an acidic intracellular compartment

makes feasible expression cloning of additional biogenic amine and amino acid vesicular transport molecules, and provides a convenient assay system for discovery of novel pharmacological agents that are specific for blockade of transport of particular biogenic amine and amino acid transmitters into secretory vesicles.

While biogenic amine transport and accumulation can be reconstituted in fibroblastic cells without secretory vesicles per se, specific neurosecretory vesicles are the critical morphological substrate for regulated, quantal secretion of accumulated neuropeptides and biogenic amine neurotransmitters from neuroendocrine cells. The potential role of CGA in secretory vesicle generation is further supported by the observation of Weihe et al., using a highly specific CGA peptide antibody as well as CGA mRNA in situ hybridization, that this protein and its mRNA is expressed at high levels in the vesicle-containing (non-basilar) keratinocyte layer of rodent and primate skin. We intend to study the role of chromogranin A as a protein potentially involved in the genesis of neurosecretory vesicles by expressing CGA in cells in culture devoid of these organelles, and ultrastructural examination following CGA expression.

Significance to Biomedical Research

Identifying regions of neuropeptide genes necessary for cell-specific expression in primary neuroendocrine cells will allow proteins that bind to these regions and differentially enhance or down-regulate neuropeptide gene transcription to be isolated and characterized. Analysis of biogenic amine/amino acid transport in reconstituted vesicular transport systems in vitro will provide a facile experimental system for development of a general chemiosmotic explanation for monoamine/amino acid accumulation in secretory vesicles.

Proposed Course of Project:

The work described above will be followed to the endpoints of identifying molecules expressed in neuroendocrine cells

that ultimately govern diversity of secretory vesicle neuropeptide and neurotransmitter content. Reconstitution of the function of these molecules in non-neuroendocrine cells will be used as assay systems for design and testing of pharmacological agents that mimick or block their action, which may be useful in probing the function of specific neuropeptide- and neurotransmitter-containing neuronal and endocrine cell populations.

Publications:

Agoston DV, Eiden, LE, Brenneman DE, Gozes, I. Spontaneous electrical activity regulates vasoactive intestinal peptide expression in dissociated spinal cord cell cultures, Mol Brain Res 1991;10:235-40.

Weihe E, Horsch D, Eiden LE, Hartschuh W. Identification of a chromogranin A-immunoreactive population of elongate endocrine-like cells in the human anal canal, Neurosci Letts 1991;130:190-4.

Iacangelo A, Grimes M, Eiden LE. The bovine chromogranin A gene: Structural basis for hormone regulation and generation of biologically active peptides, Mol Endocrinol 1991;5: 1651-60.

Erickson JD, Lloyd R, Trojanowski JQ, Iacangelo AL, Eiden LE. Sites of synthesis of chromogranins A and B in the human brain. Neuropeptides 1992;21:239-44.

Eskay RL, Eiden L.E. IL-1alpha and TNFalpha differentially regulate enkephalin, VIP, neurotensin and substance P biosynthesis in chromaffin cells. Endocrinology 1992;130: 2252-58.

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

PROJECT NUMBER
Z01 MH 02387-06LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Fine Mapping CD4/gp120 Interactions *and* SIV-infected rhesus monkeys: a neuro-AIDS model

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

PI:	Lee E. Eiden	Chief, UMCN	LCB, NIMH
	Dianne Rausch	Sr. Staff Fellow	LCB, NIMH
Others:	Pat Padgett	Chemist	LCB, NIMH
	J. Erickson	FAES Pre-Doctoral	LCB, NIMH
	D. Huddleston	Biologist	LCB, NIMH
	A. da Cunha	Visiting Scientist	LCB, NIMH
	M. Eglitis	Expert Scientist	LCB, NIMH

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LAB/BRANCH
Laboratory of Cell Biology

SECTION
Unit on Molecular and Cellular Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Bethsda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
7	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.)

The role of the CDR2- and CDR3-like domains of CD4 in binding to the HIV-1 gp120 envelope glycoprotein has been further examined with monoclonal antibodies to th CDR3-like domain and derivatized peptides from the CD4(81-92) [CDR3-like] region of CD4.Antibodies to the CDR2- and CDR3-like domain of CD4 can bind simultaneously to discrete epitopes on the CD4 molecule, yet both block gp120 binding to CD4, demonstrating separate binding sites for gp120 within the CDR2 and CDR3-like domains of CD4. CDR3- and CDR2-directed antibodies, block in vitr infection by primary/clinical pediatric isolates of HIV-1 resistant to soluble CD4. CD4(81-92) peptides block infection of some but not all of these isolates. The interaction between CD4 and the HIV-1 gp120 envelope glycoprotein involves multiple sites on both molecules, and a processive series of binding events leading to virus entry and syncytium formation. Infection of juvenile rhesus macaque monkeys with SIVsmm is a model for the AIDS dementia complex and the involvement of the central nervous system in HIV disease. The neurochemical and immunochemical sequelae of SIV infection leading to motor and cognitive deficits is under examination in the brains of SIV-infected macaques. Diffuse regional cerebrocortical astrogliosis and increased expression of somatostatin mRNA have been noted in the frontal and parietal cortices of SIV-infected monkeys. Systematic studies will reveal whether or not regional patterns of astrogliosis and neuropeptide dysregulation are markers for, or contributory to, motor and cognitive impairment accompanying SIV infection in the rhesus macaque monkey. Markers and possibly therapeutic proteins can potentially be targeted to the brain during chronic inflammatory states such as HIV infection via transduction of bone marrow cells with retroviral expression vectors, since lymphocytes, perivascular microglia, and extravasated macrophages are of hematogenous origin and may be increased in concentration in the encephalitic CNS.Retrovirally tagge bone marrow stem cells introduced into lethally irradiated donor mice can be found, albeit rarely, in the central nervous system.Attempts to increase brain targeting of retrovirally transduced hematopoietic cells in mouse models of encephalitis are underway.

Project Description:

Biospecific interaction analysis using surface plasmon resonance detection, and conventional assays of HIV infection and syncytium formation, have been used to correlate peptide and antibody epitopes on CD4 and gp120 with viral neutralization to fine-map contact regions between CD4 and gp120 involved in virus entry and virally-induced cytopathogenesis in vitro.

We hypothesize (see Eiden and Lifson, 1992, below) that both the CDR2- and CDR3-like domains of CD4 are involved in virus interaction with its receptor. These two domains may contribute equally to the processive conformational changes leading to high-affinity interaction between CD4 and gp120, but make unequal contributions to the stability of the final CD4/gp120 complex. In addition, the relative contributions of these two domains may differ between laboratory and primary/clinical isolates of HIV-1, as well as macrophagetropic and lymphocytotropic HIV-1 strains.

We are continuing to study CDR3-directed monoclonal antibodies, and are attempting to generate higher-affinity CDR3-directed MAbs, in order to map the processive binding events (vide infra) involving the CDR2- and CDR3-like domains in virus entry. Both CDR3-directed MAbs, and CD4(81-92) peptides appear to block primary/clinical, as well as laboratory isolate HIV-1 infection in vitro. A subset of these isolates were refractory to blockade with CD4(81-92) peptides. We are currently studying a variety of CD4-directed antibody and peptide reagents for a) blockade of CD4-resistant HIV-1 isolate infection, both alone and in combination, b) binding of antibodies and peptide to CD4 both before and after binding of gp120, in order to identify 'freezing' epitopes for this processive interaction and c) antibodies directed to the CDR3 region of CD4 that might be candidates for generation of anti-idiotypic antibody formation and passive immunization against HIV and SIV.

'Neuro-AIDS' describes the interaction between the brain and HIV during the course of immunodeficiency disease following lentivirus infection. These include the potential role of the brain as a viral reservoir during viral latency, the effects of HIV infection on motor

and cognitive functioning during disease, and the effects of viral infection of the brain on the hypothalamo-hypophyseal-adrenal axis and its role in the development of systemic immunodeficiency. The SIV-infected rhesus macaque is a well-established model for AIDS. We have demonstrated that it is also an animal model for neuro-AIDS: SIV-infected rhesus macaques exhibit motor and cognitive impairments during the course of SIV infection. Cognitive impairments occurred in 3/8 productively infected juvenile macaques (Murray et al., 1992). Early motor or cognitive impairment presaged a poor course of disease, since in the one year period following infection, 75% (3/4) of animals exhibiting early deficits succumbed to disease, while only 25% (1/4) of animals exhibiting late and mild motor impairments succumbed to disease. In a subsequent study, a group of juvenile rhesus macaques were inoculated with SIV simultaneously with initiation of training on a variety of neurocognitive tasks. In this paradigm, 2 of 3 animals demonstrated significant retardation in acquisition of delayed matching to sample performance and succumbed to disease within the first 6 months post-inoculation and 1of 4 of the remainder of the cohort failed to reach criterion on this test after several months of training (Rausch et al., unpublished observations). In the earlier study, virally-induced neuropathology was evident in the brains of several of the animals, but there was no apparent correlation between extent of viral lesions and cognitive impairment. Regional astrogliosis was observed in tissue blocks from the brains of all infected animals, however, suggesting that cognitive and motor impairment could be related to diffuse reactive astrogliosis causing neuronal dysregulation occuring in specific brain areas in some but not all animals with SIV encephalitis. This hypothesis is currently being tested by mapping of regional GFAP immunoreactive staining throughout the brains of cognitively impaired and unimpaired animals, and attempted concordance with markers for neuronal function, including somatostatin gene expression. Preprosomatostatin mRNA levels have previously been shown by others to be up-regulated upon acute stab wounding of rodent brain, which is also accompanied by both local and regionally diffuse increases in astrocytic GFAP staining and protein levels.

The study of viral entry into the CNS is another aspect of neuro-

AIDS currently under study. The 'Trojan horse' hypothesis of SIV/HIV infection of the CNS via entry of infected macrophages is currently favored, but investigation of this question is limited by lack of information about the physiological conditions under which macrophages enter the CNS and perivascular microglial cells mobilize at sites of inflammation and macrophage extravasation in the CNS. Studies have been initiated by Dr. Martin Eglitis in the LCB to study this problem by marking bone marrow cells of the mouse via retroviral infection to follow macrophage and microglial colonization of the brain during development and upon brain injury. Successful application of this technique in mouse may allow application to the rhesus macaque, using primate retroviral transduction and tagging of rhesus bone marrow stem cells.

Significance to Biomedical Research:

Fine mapping of CD4/gp120 interaction during HIV-1 infection, particularly differences between CD4 binding of gp120 from primary vs. laboratory, and macrophagetropic vs. lymphocytotropic strains of HIV-1 may be useful for the design of immunological and pharmacological reagents to block HIV-1 infection and cytopathogenesis during specific stages of viral disease, and for rapid 'virus-typing' to determine whether a particular CD4-based therapy is appropriate for a given HIV-infected individual.

The SIV-infected rhesus macaque represents an animal model both for further understanding the role of the brain as a viral reservoir in HIV disease, for identifying mechanisms of neuropathogenesis in HIV encephalopathy, and for pre-clinical evaluation of agents for the treatment of AIDS dementia complex.

Proposed Course of Project:

We intend to identify specific neural, viral and immune components of the CNS response to SIV infection and potential neurochemical, immunochemical and cytokine candidates for mediation of motor/cognitive impairment caused by SIV infection. We will examine the role of the brain in viral disease course, and the importance of the brain in intra-host generation of lymphocytopathic quasi-species of the virus, by comparing disease

course, brain infection, and motor/cognitive impairment following inoculation of rhesus macaques with macrophage- and lymphocytotropic clones of SIV. High affinity monoclonal antibodies to the CDR3 region of CD4, as well as mouse/rhesus CD4 chimeras, will be developed for in vitro evaluation and potentially for passive immunotherapy as well as anti-idiotype generation and virus neutralization in vivo. Additional CD4-based peptides will be evaluated for potential pre-clinical treatment of SIV-infected rhesus macaques. A rhesus macaque model for perinatal AIDS is currently being investigated. Extension of retroviral infection of bone marrow cells for tagging of brain-targeted macrophage and microglial cells from rodent to primate if the results obtained in the former system apear to warrant development of this approach.

Publications:

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Murray EA, Rausch D M, Lendvay J, Sharer L, Eiden LE. Cognitive and motor impairments associated with SIV infection in rhesus monkeys. Science 1992;255:1246-9.

Lifson J D, Eiden LE. HIV interactions with CD4: a continuum of conformations and consequences. Immunol Today 1992;13: 201-6.

Rausch D M, Lifson J D, Padgett M P, Chandrasekhar B, Lendvay J, Hwang K M, Eiden L E , CD4(81-92)-based peptide derivatives: structural requirements for blockade of HIV infection, blockade of HIV-induced syncytium formation, and virostatic activity in vitro, Biochem Pharmacol 1992; in press.

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

PROJECT NUMBER
Z01 MH 02396-06LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

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 on Neurobiology	LCB, NIMH
Others:	Paul M. Byrne	Biomedical Engr Technician	LCB, NIMH
	Nobuko Tasaki	Guest Researcher	LCB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Laboratory of Cell Biology

SECTION
Unit on Neurobiology

INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Bethsda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	1	2

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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.)

During the early part of this fiscal year, we began to investigate the process of heat production associated with impulse conduction in the myelinated nerve fibers. Because of the rapidity and the smallness of the amount of thermal energy liberated by these rapidly conducting nerve fibers, the classical thermal detector, namely, a thermopile-galvanometer system, is known to be inadequate to determine the time-course of heat production in these fibers (see Keynes and Ritchie; J. Physiol., Vol. 210, 29P, 1970) Using thin film of poly(vinylidene fluoride) we have constructed a sensitive thermal detector with a time-resolution of about one millisecond. [Note that the time-resolution achieved by the classical detector was 50-100 milliseconds.] We have succeeded in determining the time-course of the heat generated by the amphibian sciatic nerve at 5°C. The results obtained have been interpreted based on the divalent-univalent cation-exchange theory developed previously in this laboratory.

In December of 1991, we started investigating the origin of the rapid structural changes in the garfish olfactory nerve associated with the excitation process. By combining our devices for detecting changes in the optical properties of extrinsic dye molecules with the piezo-electric device, we have recorded several types of non-electrical manifestations of the excitation process in the olfactory nerve. We have found that, at the site of electric stimulation, the lateral expansion of the nerve fibers (i.e. swelling) starts simultaneously with, or precedes, the onset of many optical changes. The results of these measurements strongly suggest that a rise of the water-content in the superficial layer of the nerve fiber is at the base of the observed optical signals.

PROJECT DESCRIPTION

Objective

The objective of this research is to elucidate the function of the nervous system examining non-electrical manifestations of excitation processes. In recent years, we have succeeded in greatly improving the time-resolution of the heat sensors by using thin film of synthetic pyroelectric polymer, poly(vinylidene fluoride). By use of these improved heat sensors, we wish to analyze the process of energy metabolism in various tissues in the nervous system. [Note that the biochemical methods of studying the rate of energy metabolism have only limited time-resolution.] We also wish to apply the method of recording rapid mechanical changes in various parts of the nervous system in order to clarify the involvement of water molecules in nervous activities. We also employ various optical methods for studying non-electrical manifestations of excitation processes.

In September of 1991, we began our study of thermal changes in amphibian myelinated nerve fibers for the purpose of elucidating the energy metabolism in these rapidly conducting fibers. Previous investigators, employing classical thermopile-galvanometer systems for detection, have succeeded in demonstrating the existence of heat production, but they failed to determine its time-course. We wish to clarify the origin of the nerve heat by directly determining the time-source using our poly(vinylidene fluoride) detector. We have modified the design of our detector to make it suitable for recording from a relatively long object functioning at low temperatures. (This project turned out to be quite successful.)

In January of 1992, we started analyzing the origin of various optical changes in nerve fibers associated with the excitation process. Since we have previously clarified the physico-chemical nature of the process of rapid swelling in nerve cells and fibers, we have good ground to believe that

optical changes in nerve fibers may be directly or indirectly related to the enhancement of the water-content in the superficial gel layer of the nerve fibers. An additional incentive to study this problem was the fact that the P.I. was invited to participate in international symposia (held in Japan and also in England) dealing with optical changes in the nervous system. We chose to examine birefringence changes, dye-absorption changes, changes in light scattering and extrinsic fluorescence in the garfish olfactory nerve. Our objective was to establish a correlation between the time-course of nerve swelling and those of various optical signals evoked by nerve stimulation. (At present, this research project is close to a successful conclusion.)

Methods

To determine temperature changes associated with excitation of myelinated nerve fibers, we constructed thermal detectors with a large (3X8 mm^2) heat-sensitive area using pyroelectric film purchased from Pennwalt Corp. We coated all the electric parts of the detectors with "Uralane" which makes the coated surface strongly water-repellent. The piezoelectric device and optical setups, constructed previously in this laboratory, were employed in our investigation of the origin of rapid optical changes in the nerve fibers.

Major Findings

Our investigation of the time-course of heat production associated with a single impulse conducted along an amphibian sciatic nerve has revealed that, at about $5^{o}C$, the temperature rise following nerve stimulation is roughly 0.4 micro-degree, centigrade. As in nonmyelinated nerve fibers, roughly three quarters of the heat generated was re-absorbed by the nerve during the second (negative) phase. Although our analysis of the heat generated by the sciatic nerve was complicated by the existence of several fiber types (alpha,

beta, etc.), we have inferred from the present analyses that the mechanism of heat production in the myelinated nerve fiber is similar to that in the nonmyelinated nerve fiber, the positive heat production being associated by an cation-exchange process involving Ca-ions in the superficial layer of the nerve fiber.

Using the garfish olfactory nerve, we have demonstrated that the time-course of birefringence changes associated with the excitation process coincides nearly perfectly with the swelling curve taken directly from the site of optical recording. Since the birefringence change is considered to be an expression of a transient decrease in the form-birefringene of the nerve fibers at rest, the coincidence between these two non-electrical signals strongly suggests that the invasion of water molecules into the superficial layer of the nerve fiber brings about both swelling and birefringence changes. Furthermore, we have seen that the light scattering changes and the meropcyanine dye-absorption signals also have the same time-course as the swelling of the nerve fibers. When very large dye molecules (such as bicarbocyanine) were used in these studies, the dye absorption or fluorescence signals were found to lag behind that of the swelling curve. Based on these experimental findings, we have concluded that the enhancement of the water-content in the superficial layer of the nerve fiber is at the base of all the optical mechanical signals observed during excitation of the nerve fiber.

Significance in Biomedical Research

Our studies on non-electrical manifestations of excitation processes have clarified several basic aspects of the physiologicl events leading to the propagation of impulses along nerve fibers. A proper understanding of the processes of nerve excitation and synaptic transmission is essential for improving treatments of diseases of the nervous system.

Publications

Tasaki I, Byrne P. M. Thermal and mechanical responses of the amphibian skin to nerve stimulation Jpn J Physiol 1991;41:567-76.

Tasaki I. Bistability of the nerve membrane: mechanical and thermal changes in nerve fibers during excitation. Biomed Res 1991;12-2:19-20.

Tasaki I, Byrne P.M. Discontinuous volume transitions in ionic gels and their possible involvement in the nerve excitation process. Biopolymors 1992;32:1019-23.

Tasaki I, Byrne P.M. The origin of rapid changes in birefringence, light scattering an dye absorption associated with excitation of nerve fibers. eds. Academic Publications: The Frontier of Optical Neurobiology, Tokyo, In Press.

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

PROJECT NUMBER
Z01 MH 02497-03 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Functional Organization of the Basal Ganglia

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

PI:	C.R. Gerfen	Research Biologist	LCB, NIMH
Others:	J. Marcus	Biologist	LCB, NIMH
	R. Paletzki	Biologist	LCB, NIMH
	H. Steiner	Visiting Fellow	LCB, NIMH
	L. Nisenbaum	Guest Researcher	LCB, NIMH

COOPERATING UNITS (if any)
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LAB/BRANCH
Laboratory of Cell Biology

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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS: 4.0	PROFESSIONAL: 2.0	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.)

Research in this group studies the basal ganglia, a major neural system through which the cerebral cortex affects behavior. The major component of the basal ganglia, the striatum, receives inputs from most of the cortex. Three major lines of research are followed: 1) Neuroanatomical mapping of basal ganglia connections, 2) characterization of striatal neurons in terms of their connections, and expression of repetoires of neurotransmitter receptors subtypes, signal transduction systems, and peptides, and 3) pharmacologic manipulations of dopaminergic, cholinergic and peptidergic systems to determine the functional organization of the striatum using quantitative *in situ* hybridization histochemistry. Neuroanatomical studies reveal a hierarchy of functional compartments within the striatum that reflect parallel processing of cortical information. For example, the two major output systems of the striatum, the striatonigral and striatopallidal pathways respectively express D1 and D2 dopamine receptor subtypes. In normal behavior there is a delicate balance between these two pathways, which regulate excitatory and inhibitory activity of the major output systems of the basal ganglia. In Parkinson's disease, the normal balance is disrupted and the striatopallidal pathway appears to become overactive. Studies from this research group have provided insights into the cellular and molecular mechanisms underlying basal ganglia dysfunction.

Project Description:

Research in the Neuroanatomy Group is focussed on the functional organization of the basal ganglia with the goal of understanding how the striatum processes cortical information to affect behavior. Three major lines of research are followed: 1) Detailed neuroanatomical mapping of basal ganglia connections, 2) characterization of striatal output neurons in terms of their connections, and expression of repetoires of neurotransmitter receptors, signal transduction systems, and neurotransmitters/peptides, and 3) pharmacologic manipulations of dopaminergic, cholinergic and peptidergic systems to determine the functional organization of the striatum.

Progress made in 1992:

In prior studies our studies have established that the D1 and D2 dopamine receptor subtypes are localized to segregated striatal output systems. As a result dopamine appears to function to oppositely modulate the cortical information processed through the basal ganglia. During the past year studies have been directed at interactions that occur between striatal neurons when both D1 and D2 dopamine receptors are activated concurrently. Results show that synergism occurs as a result of the segregation of D1 and D2 receptors to separate neurons, not as a result of their coexistence in single neurons. One model of such interactions involves the effects of cocaine and amphetamine. Using this model, a causal reciprocal relationship between the opiate peptide dynorphin and the postsynaptic effect of dopamine on striatal output neurons has been demonstrated.

Significance relative to the goals of the NIMH, IRP:

Dysfunction of dopaminergic action within the striatum causes a broad spectrum of clinical disorders from the bradykinesia of Parkinson's disease where there is a loss of dopamine function to the uncontrolled movements, psychoses and psychostimulant abuse that results from excess dopamine action. Our studies have established a model that suggests such disorders result from imbalances in the regulation of the striatopallidal and striatonigral output pathways. We provide a means of assaying the pharmacologic manipulation of these pathways using quantitative in situ hybridization histochemistry to develop strategies of therapeutically managing dopaminergic imbalance. For example, we have demonstrated that in the rat model of Parkinson's disease, the loss of dopamine to the striatum results in a specific upregulation of the striatopallidal pathway. This upregulation is thought to be the cause of bradykinesia. In our model, L-DOPA treatments overcome this imbalance not by reversing the upregulation of the striatopallidal pathway, but rather by upregulating the striatonigral pathway. It is our hypothesis that the long term affects of such treatment produces tardive dyskinesia. On the other hand, if a D2 dopamine agonist is used to treat dopamine deafferented animals, there is a select reversal of the lesion induced imbalance. Our studies suggest further that the treatment schedule is critical for the ameliorating affects of a D2 agonist and that this treatment schedule is markedly different than would be prescribed for the use of D1 drugs. Moreover, we are extending this strategy to examine psychostimulant abuse models.

Publications:

Gerfen CR. The neostriatal mosaic: multiple levels of compartmental organization, Trends Neurosci 1992;15:133-9.

Gerfen CR. The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia, Annu Rev Neurosci 1992;15:285-330.

Gerfen CR, Engber TM. Molecular neuroanatomical mechanisms of Parkinson's disease: a proposed therapeutic approach, Neurol Clin 1992;10:435-49.

Hurd YL, Brown EE, Finlay JM, Fibiger HC, Gerfen CR. Cocaine self-administration differentially alters mRNA expression of striatal peptides, Mol Brain Res 1992;13:165-70.

Engber TN, Susel Z, Kuo S, Gerfen CR, Chase TN. Levodopa replacement therapy alters enzyme activities in striatum and neuropeptide content in striatal output regions of 6-hydroxydopamine lesioned rats, Brain Res 1991;552:113-8.

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

PROJECT NUMBER
Z01 MH 02498-03 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Regulation of Gene Expression in the Brain

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

PI:	W.S. Young	Medical Officer	LCB, NIMH
Others:	E.A. Shepard	Biologist	LCB, NIMH
	C. LeMoine	Guest Researcher	LCB, NIMH
	N. Ostrowski	Sr. Staff Fellow	LCB, NIMH
	S. Bachus	IRTA Fellow	LCB, NIMH
	J. Finkelstein	Guest Researcher	LCB, NIMH
	D. Bradley	Guest Researcher	LCB, NIMH
	A. Hartman	Guest Researcher	LCB, NIMH

COOPERATING UNITS (if any)
Neuropathology Lab, Johns Hopkins Sch of Med; Dept. Pathology, Univ. Arizona Sch of Med; Lab of Biochemical Genetics, NHLBI

LAB/BRANCH
Laboratory of Cell Biology

SECTION

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
6.5	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.)

Expression of genes encoding neuropeptides and enzymes in the brain, with emphasis on the hypothalamus, are being studied. We isolated the rat vasopressin and oxytocin genes and are continuing our transgenic mice experiments (that enabled expression of oxytocin in a tissue and physiologically specific fashion) with promoter-mutated transgenes. We are also studying the novel POU protein, RHS2 (Brain-4) whose full-length cDNA we cloned last year. We are pursuing double simultaneous in situ hybridization studies to determine potentially regulated genes. We are also mapping the sites of expression of the four class III POU proteins to further our understanding of their roles, especially in advance of initiated homologous recombination studies. We have mapped the sites of expression during development of the four thyroid hormone receptor subtypes and identified sites of action responsible for defects encountered in fetal thyroid hormone deprivation. We have also examined the sites of expression of two vasopressin and two somatostatin receptor subtypes and are currently analyzing these data. Mapping of gene expression in the human hypothalamus continues, with analysis of vasopressin, oxytocin, and LHRH completed with opioids and tachykinins in progress. Regulation of galanin expression in the paraventricular and supraoptic nuclei by the arcuate nucleus is being studied through the use of monosodium glutamate, a toxin.

Project Descriptions

Our group investigates the regulation of gene expression in the mammalian brain, with emphasis on the hypothalamus. We have isolated the rat genes for vasopressin and oxytocin and used them to make constructs that express oxytocin in transgenic mice in a cell- and physiologic-specific way. We have made mutations in the promoter of the oxytocin promoter that decrease thyroid, estrogen and retinoic acid hormone influences *in vitro* and are currently examining these mutated transgenes *in vivo*. We are using molecular cloning, mouse embryo DNA injections and transfers, *in situ* hybridization, and immunohistochemistry in this portion of our studies. This work is done with E. Shepard, K. Reynolds, and A. Zimmer.

We are characterizing a POU protein, RHS2 (Brain-4), that we cloned. Double simultaneous *in situ* hybridizations have colocalized RHS2 with a variety of neuropeptide transcripts and we have shown it to be an octomer binding protein. We are pursuing gel shift assays with other gene fragments based on our double simultaneous *in situ* hybridizations. A. Hartman is expressing the RHS2 in bacteria and will use it for generating antibodies and various studies of protein-protein-DNA interactions. The promoter region of Brain-4 has been coupled with a luciferase reporter and potential cis-acting elements are under examination using transient transfections. With Drs. Y. Hara and M. Nirenberg of the Laboratory of Biochemical Genetics, NHLBI, A. Hartman, D. Bradley and I are mapping the distribution of expression during development of the mouse of all four class III POU genes. We are initiating homologous recombination experiments with Drs. Hara, Nirenberg, and Zimmer to study the role of Brain-4.

The developmental expression patterns of the four thyroid hormone receptor isoforms in the rat are being studied by David Bradley using *in situ* hybridization, northern and PCR analyses. We are also studying the developmental, physiological and regional patterns of expression of the vasopressin and somatostatin receptor subtypes in the rat using *in situ* hybridization. This latter work is being pursued by Dr. Ostrowski and myself in collaboration with Drs. Lolait and O'Carroll of the LCB.

S. Bachus is completing her studies of the regulation of galanin expression in the rat PVN and SON by the arcuate nucleus. She has used a variety of electrolytic and chemical lesions in combination with *in situ* hybridization.

We are continuing our studies of transcripts in the human hypothalamus by *in situ* hybridization. These studies are done in collaboration with D.L. Price and L.C. Walker (Neuropathology Laboratory, Johns Hopkins University School of Medicine) and N. Rance (Dept. Pathology, Univ. Arizona School of Medicine).

Significance to Biomedical Research

The significance of our research lies in the furthering of our knowledge of how various systems in the brain and, in particular, the hypothalamus, respond to physiological perturbations. The hypothalamus is a gateway for integration of information about the internal (e.g., stresses) and external environments. Our methods, especially *in situ* hybridization, provide high resolution snapshots of gene activity under various situations and explain certain pathological events. For example, we have shown thyroid hormone receptors in the developing eye, ear and nose that explain certain defects that arise from congenital hypothyroidism. Our studies of the transacting factor POU proteins will provide further insights into the regulation of developmental patterns. In addition, the technical advances for *in situ* hybridization that we have developed and distributed have helped other laboratories on this campus and elsewhere pursue their studies.

Proposed Course of Project

The proposed course of our research will concentrate most heavily on understanding the regulation of vasopressin and oxytocin gene expression, as well as that of the POU genes. We will attempt to understand how the latter's expression may influence vasopressin, oxytocin or other co-expressed genes. We have isolated the human counterpart of RHS2 and localized it to the X chromosome. We will try to learn if this gene is involved in any X-linked defects and where it is expressed in the human. We are beginning to make the construct necessary to knock-out the mouse's counterpart by homologous recombination, Brain-4, to study its role directly.

Publications

Rance NE, Young WS III. Hypertrophy and increased gene expression of neurons containing neurokinin-B and substance-P messenger ribonucleic acids in the hypothalami of postmenopausal women, Endocrinology 1991;128:2239-47.

Young WS III, Lightman SL. Chronic stress elevates enkephalin expression in the paraventricular and supraoptic nuclei and modifies opioid receptor binding in the median eminence and posterior pituitary of the rat. In: Kvetnansky R, McCarty R, Axelrod J, eds. Stress: Neuroendocrine and molecular approaches. New York:Gordon and Breach Science Publishers, 1992;221-34.

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Aksentijevich S, Whitfield HJ Jr, Young WS III, Wilder RL, Chrousos GP, Gold PW, Sternberg EM. Arthritis-susceptible Lewis rats fail to emerge from the stress hyporesponsive period, Dev Brain Res 1991;65:115-8.

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Levy A, Matovelle MC, Lightman SL, Young WS III. The effects of pituitary transection, hypophysectomy and thyroid hormone status on insulin-like growth factor 2, growth hormone releasing hormone, and somatostatin mRNA prevalance in rat brain, Brain Res 1992;579:1-7.

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Bradley DJ, Towle HC, Young WS III. Spatial and temporal expression of α and β thyroid hormone receptor mRNAs, including the $\beta 2$ subtype, in the developing mammalian nervous system, J Neurosci 1992;12:2288-302.

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Gouras GK, Rance NE, Young WS III, Koliatsos VE. Tyrosine-hydroxylase-containing neurons in the primate basal forebrain magnocellular complex, Brain Res 1992;in press.

Young WS III. Expression of the oxytocin and vasopressin genes, J Neuroendocrinology 1992;in press.

Belenky M, Castel M, Young WS III, Gainer H, Cohen S. Ultrastructural immunolocalization of rat oxytocin-neurophysin in transgenic mice expressing the rat oxytocin gene, Brain Res 1992;583:279-86,

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

PROJECT NUMBER
Z01 MH 02591-01 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Identification of a human cDNA that suppresses the tumorigenicity

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

PI: Maribeth V. Eiden Sr. Staff Fellow LCB, NIMH
Others: Linda MacArthur IRTA FELLOW LCB, NIMH

COOPERATING UNITS (if any)

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

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☐ (a1) Minors
☐ (a2) Interviews

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

Expression of the human cDNA encoding the intermediate filament protein vimentin induces reversion of nitrosylmethylurea (NMU) transformed Syrian hamster kidney fibroblasts (NMU 34m cells) to a normal phenotype as assessed by morphology, anchorage independent growth parameters, and suppression of the tumorigenic phenotype and is apparently required for maintenance of the normal phenotype in BHK SN-10 cells. NMU 34m cells are transformed by inactivation of a tumor suppressor gene. BHK cells that are transformed as a result of infection by a tumor virus or by the introduction of an activated human oncogene also exhibit tumorigenic properties similar to NMU 34m cells. Expression of the human vimentin cDNA is not sufficient to revert virally or oncogene transformed BHK cells. Therefore vimentin functions in a manner analogous to a tumor suppressor gene in that its suppressive effects are restricted to cells transformed by recessive mechanisms.

PHS 6040 (Rev. 5/92)

Project Description:

Transformation of the baby hamster kidney cell line BHK SN-10 by chemical carcinogens such as nitrosylmethylurea (NMU) is mediated by the loss of a gene product critical for the suppression of malignant transformation. BHK SN-10 fibroblasts are well suited to the identification of cellular genes important in appropriate regulation of cell growth, whose expression is critical in suppressing malignant transformation. Malignant transformation of BHK SN-10 cells by NMU is the result of the inactivation of a cancer suppressor gene and results in the simultaneous acquisition of several phenotypic traits: changes in cell morphology and growth properties (anchorage independent growth), disruption of actin cytoskeleton, the increased ability to form tumors in animals, and loss of inhibition of angiogenesis. All of these phenotypic traits are concommitantly suppressed when NMU transformed hamster cells are fused to BHK SN-10. Normal human fibroblasts can also suppress the transformed phenotype of NMU cells indicating that a human gene products(s) can complement the genetic defect in NMU transformed hamster cells. In contrast when polyoma virus or ras oncogene transformed BHK cells are fused to normal BHK cells or normal human fibroblasts the tumor phenotype is dominant.

We had previously determined that expression of the human cDNA encoding the intermediate filament vimentin is sufficient to induce reversion of NMU 34m cells to a flat, elongated, contact inhibited, anchorage dependent, nontumorigenic phenotype similar to that of the parent BHK SN-10 cells. Expression of human vimentin cDNA in polyoma or ras transformed BHK cells did not induce reversion to the nontransformed phenotype. These findings suggest that the vimentin gene is similar to other tumor suppressor genes in that it's function is restricted to cells that are

transformed by recessive mechanisms.

Significance to Biomedical Research:

A large number of dominant effectors of cellular transformation such as viruses and oncogenes have been identified and functionally characterized. Considerably less success has been made in identifying and characterizing the function of recessive mediators of cellular transformation. We have identified and are now attempting to understand the mechanism underlying vimentin induced reversion of the tumorigenesis.

Proposed Course:

We are planning to continue our analyses of the mechanism of the vimentin induced reversion of NMU 34m cells. We will be molecularly characterizing the vimentin gene endogenous to NMU 34m cells and comparing it to the gene found in BHK SN-10 cells.

Publications:

None.

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

PROJECT NUMBER
Z01 MH 02592-01 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Viral and Cellular Factors Governing Retroviral Infection

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

PI:	Maribeth V. Eiden	Sr. Staff Fellow	LCB, NIMH
Others:	Carolyn A. Wilson	NRC Fellow	LCB, NIMH
	Karen B. Farrell	Biologist	LCB, NIMH
	Martin Eglitis	Special Expert	LCB, NIMH
	Lawrence Mahan	Res. Biologist	LCB, NIMH
	Jennie Warsowe	Summer IRTA	LCB, NIMH
	Jon Marsh	Sr. Staff Fellow	LMB, NIMH
	Eva Mezey	Visiting Sci	CNB, NINDS

COOPERATING UNITS (if any)
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4.0	2.75	1.25

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☐ (a1) Minors
☐ (a2) Interviews

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

We are interested in improving the efficiency of retroviral mediated gene transfer. Retroviral vectors provide the most efficient means of stably expressing genes in cells both *in vitro* and *in vivo*. However not all types of cells are efficiently infected by retroviruses. We have developed an approach that allows us to infect cells that are refractory to retroviral infection. This approach is predicated on identifying and functionally characterizing the receptors used by retroviral vectors to gain entry into cells and mapping the regions of the cellular receptor which are critical for retrovirus-receptor interaction. We have used three different types of recombinant vectors, two murine based vectors (ecotropic and amphotropic) and a gibbon ape vector (GaLV) in our analyses. 1. We have cloned a murine ecotropic receptor and precisely mapped the amino acid residues that are critical for binding and entry of ecotropic vectors. 2. A segment of human DNA which confers susceptibility to amphotropic virus infection (a putative amphotropic receptor) has been functionally identified and is in the process of being isolated and molecularly characterized. 3. We have used oligomeric probes derived from the GaLV receptor sequence to examine spatial and temporal expression of receptor mRNAs in the rat by *in situ* hybridization. If the GaLV receptor is restricted to a certain cell type, it may be possible to target genes using GaLV retroviral vectors to a particular tissue. We have also initiated studies to functionally map the GaLV binding region of the receptor.

Project Description:

The research focus of the Unit on Molecular Virology has been directed towards understanding the molecular mechanisms underlying retroviral infection. It is the interaction between the envelope of the retrovirus and the receptor on the surface of the target cells that initiates the infection process. The envelope protein of the retrovirus binds to a site on the receptor and then fuses with the cell membrane. Mapping the sites on the envelope and receptor that participate in these functions is a necessary first step towards overcoming receptor-mediated blocks to retroviral infection and ultimately to the development of retroviral vectors that can be targeted to specific types of cells.

Analysis of Ecotropic Retrovirus-Receptor Interactions:

Karen Farrell and Jennie Warsowe have cloned a nonfunctional ecotropic virus receptor from the MDTF (*Mus dunni* tail fibroblast) cell line and we have determined that by changing a valine residue to an isoleucine residue in the third extracellular domain of this multimembrane spanning cell surface protein we can convert this MDTF protein into a functional ecotropic receptor. In addition Carolyn Wilson has found that this nonfunctional MDTF receptor can be rendered functional by the removal of N-linked carbohydrate moieties. We are initiating experiments in collaboration with L. Mahan, to further resolve the functional consequences that these conformational changes in the receptor have on ecotropic virus binding and infection.

Carolyn Wilson and Jon Marsh have determined that treatment of *ras*-transformed murine cells with chloroquine, an agent that raises endosomal pH, blocks ecotropic murine virus entry but not virally-induced syncytium formation. Thus envelope-mediated entry, which requires low pH, appears to occur

within a cellular endocytic vesicle. In contrast, virally-mediated cell-cell fusion or syncytium formation is pH independent. Therefore these two processes are not co-dependent and may map to dissociable epitopes on the viral envelope.

Analysis of Amphotropic Retrovirus-Receptor Interactions:

Martin Eglitis is currently engaged in a program to clone the gene for the human receptor for amphotropic retroviruses. Because Chinese hamster ovary cells (CHO-K1) are resistant to infection by amphotropic retroviruses, they provide a useful model for study of the nature of the block to infection. As an initial step in isolating the human gene responsible for susceptibility to infection with amphotropic retroviruses, a CHO-K1 cell-derivative (CHO18) susceptible to amphotropic retrovirus infection has been generated by introducing genomic DNA from infectable human cells. CHO18 are as efficiently infected by amphotropic retroviruses as control NIH 3T3 fibroblasts. Furthermore, this acquired susceptibility is specific for retroviral vectors bearing an amphotropic envelope.

To help determine if the human DNA in CHO 18 cells encodes an amphotropic receptor, fusion hybrids have been generated between CHO18 cells and bovine kidney cells (MDBK). MDBK cells, like CHO K1 cells, are resistant to amphotropic retrovirus infection. However, CHO K1 but not MDBK cells can be rendered sensitive to amphotropic virus infection by pretreatment with tunicamycin, an inhibitor of N-linked glycosylation. Putative hybrid cells have been isolated which are infected as well as CHO18 cells, thus the susceptibility to infection of CHO18 cells is most likely due to the presence of a true amphotropic receptor and not a consequence of nonspecific factors such as the expression of an inhibitor of glycosylation.

Two approaches are being used to identify the human gene present in CHO18 cells which encodes the amphotropic

receptor. A genomic library has been constructed from CHO18 cells and clones containing human sequences have been isolated and are in the process of being molecularly characterized. The second approach involves the use of polymerase chain reaction methodology to clone CHO18 cDNAs which are homologous to the Gibbon ape leukemia virus (GaLV) receptor (GLVR-1). The rationale for this approach is based on the finding that a human gene that is highly related to the previously cloned GaLV receptor has been localized to human chromosome 8. The human amphotropic receptor has been functionally mapped (using human hamster somatic hybrids) to human chromosome 8. Therefore it is conceivable that the amphotropic receptor is a member of the GaLV receptor family. A probe derived from sequences conserved between several species' GaLV receptors has been used to hybridize to Southern and Northern blots from CHO18 cells. CHO18 cells have been found to contain extra copies of a gene homologous to GLVR-1. Furthermore, preliminary Northern blot data suggest that a gene with homology to GLVR-1 is also over-expressed in CHO18 cells.

Analysis of GaLV-Receptor Interactions:

The GaLV cell surface receptor homolog expressed in NIH 3T3 murine cells does not function as a GaLV receptor. The human GaLV receptor has been previously cloned. Expression of the human GLVR-1 cDNA in NIH 3T3 cells allows for relatively inefficient infection of NIH 3T3 cells by GaLV. Carolyn Wilson and Karen Farrell have cloned a functional GaLV receptor from a murine fibroblast cell line derived from *M. molossinus*(MMF). Comparison of the NIH 3T3, MMF and human GaLV receptor cDNAs has suggested two regions that may be involved in mediating efficient GaLV entry into NIH 3T3 cells. One region, present in the fourth extracellular domain is important in GaLV binding. A second cytoplasmic region (found only in the NIH 3T3 and MMF cDNAs) may interact with murine cytoplasmic augmenting factors. Differences between the murine and human cDNA's in this second region could account for the inefficient functioning of the human receptor cDNA in murine cells. We are currently introducing

the MMF GaLV receptor into NIH 3T3 cells to determine if we can improve the efficiency of GaLV infection of these cells relative to NIH 3T3 cells expressing the human GaLV receptor.

Oligomeric probes have been designed and synthesized for in situ hybridization histochemistry analysis of fetal and adult rat tissues. Preliminary results of experiments conducted by Martin Eglitis in collaboration with E. Mezey indicate that the rat gene homologous to the human GaLV receptor is not ubiquitously expressed. Some tissues, such as spleen, have small numbers of cells which express high levels of the GaLV receptor homolog transcript. Furthermore, there are changes in the tissue distribution of gene expression during development. In the fetus, cells expressing significant levels of the putative GaLV receptor can be identified in the developing somites along the spine, as well as in cells lining the brain ventricals in the region of the choroid plexus. These in situ analyses are currently being combined with indirect immunofluorescence assays using antibodies to specific cellular antigens in an effort to identify the specific cell types expressing the rat GaLV receptor homolog.

Significance to Biomedical Research:

Retroviral mediated gene transfer into humans has moved from the theoretical to the practical with the recent FDA approval of several human gene transfer protocols. Retroviral vectors have been demonstrated to be a safe and efficient means of introducing genes into some lymphocytes, hepatocytes and tumor cells. However, other cell populations, such as human hematopoietic stem cells and muscle cells, are not efficiently infected. To improve retroviral gene delivery to resistant cell types it is first necessary to understand what factors influence viral susceptibility. We have developed an approach that allows us to identify the viral/cellular component that is responsible for resistance to infection and then to modify that component so as to overcome resistance to infection.

Proposed Course:

We intend to design recombinant virions that will efficiently transduce genes into cells that are resistant to infection by currently used retroviral vectors. In addition we are attempting to further characterize the specific determinants on viral envelopes and their respective cell receptors that are necessary for mediating viral entry into target cells.

Publications:

Wilson CA, Eiden MV. Viral and cellular factors governing hamster cell infection by murine and gibbon ape leukemia viruses, J Virol 1991; 65:5975-5982.

Wilson CA, Marsh J, Eiden MV. The requirements for viral entry differ from those for virally-induced syncytium formation in NIH 3T3/DTras cells exposed to Moloney murine leukemia virus, J Virol, in press.

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

PROJECT NUMBER
Z01 MH 02594-01 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Signalling Mechanisms in CNS Development

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

PI:	Andreas Zimmer	Visiting Associate	LCB, NIMH
Others:	U. Eisel	Visiting Associate	LCB, NIMH
	M. Holmgren-Konig	Biologist	LCB, NIMH
	A. Kurtz	Guest Researcher	LCB, NIMH
	K. Reynolds	Biologist	LCB, NIMH
	M. Riddick	Biologist	LCB, NIMH
	R. Cohen	Senior Surgeon	LCB, NIMH

COOPERATING UNITS (if any)
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☐ (a1) Minors
☐ (a2) Interviews

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

Research conducted in the Unit on Developmental Biology is aimed towards the elucidation of molecular mechanisms regulating the development of the central nervous system (CNS). The CNS develops through an orderly series of events that are governed by the genetic program and epigenetic signals. The anteroposterior and dorsoventral CNS axis is determined during gastrulation and early stages of neurogenesis. Subsequent steps involve proliferation of neuronal precursor cells, cell migration, axon outgrowth and the establishment of synaptic connections. The spatial and temporal pattern of these events are characteristic for each neuronal structure. Transgenic and embryonic stem cell technologies are employed to study the interaction between epigenetic signals and the genetic program in the development of the central nervous system.

Project Description:

Research in this unit is aimed towards the elucidation of the molecular mechanisms involved in the regulation on CNS development. We use transgenic mice to study the developmental regulation of genes that are potentially involved in the transduction of positional and spatial information in vivo. Transgenic and chimeric mice are also utilized to interfere with synaptic functions. The goal is a better understanding of the molecular processes which result in the manifestation of neuronal identity and the establishment and maintenance of neuronal connections. Three major routes are followed: (i) analysis of the role of retinoids and retinoic acid receptors during early neurogenic stages; (ii) expression of the tetanus toxin gene under the control of neuron specific promoters in transgenic mice as a means to disrupt neuronal communication; (iii) creation of mutations in neuronal genes by homologous recombination and "gene trapping."

Several advances were made during the last year.

1) Transgenic mice carrying a bacterial ß-galactosidase gene (lacZ) under the control of the retinoic-acid-receptor-ß-2 (RARß2) gene promoter were used to study the effects of retinoic acid (RA) on the transcriptional activity of this promoter in vivo. Administration of low doses of RA was highly teratogenic on day 7.5 but not at later stages. Effected embryos exhibited severe defects in the craniofacial region, the heart, and the anterior neural tube. We found rapid induction of ß-galactosidase within 4 hours after maternal administration of low RA doses at gestation day 7.5 in the cranial mesoderm of gastrulating embryos. These studies suggest that mesoderm transformation, possibly by ectopic induction of the RARß2 gene, plays an important role in the observed terotogenic effects of RA. RA treatment at day 8.5 to 10.5 induced expression of the transgene in a

segmented pattern reminiscent to that of pair rule genes in the forebrain, midbrain, and hindbrain. These data strongly suggest that the anterior regions of the CNS are truly segmented. The characteristic appearance of transient bulges during neural tube development has been interpreted as an indication of an intrinsic segmented structure. However, this hypothesis remained controversial because supportive molecular and cellular evidence existed only in the hindbrain region. Our transgenic mouse lines should provide useful substrates for further investigations of the molecular mechanisms underlying the segmentation of the CNS.

2) The 5'-untranslated region of the RARß2 gene contains several short open reading frames. The possible role of these open reading frames for the regulation of the RARß2 gene expression was analyzed. We found that the 5'-untranslated region was important for the high level of expression. Point mutations which effected only single open reading frames had little or no effect in vitro translation assays or in tissue culture cells. However, we found ß-galactosidase activity in the telencephalon and the heart muscle of several different transgenic lines derived with these constructs while the wild type construct was never active in these tissues. These results demonstrate that the open reading frames play an important role in the regulation of the tissue specific expression pattern of the RARß2 gene. Several genes which are supposed to be involved in the regulation of cell growth and differentiation have similar open reading frames in the 5' untranslated region. Our results indicate for the first time a possible role for this characteristic feature and suggest that a common factor might act on these genes to regulate their tissue specific expression at the posttranscriptional level.

3) Tetanus toxin is produced by Clostridium tetani. Upon uptake into neurons it inhibits the calcium-stimulated release of neurotransmitter by a yet unknown mechanism. The toxin is not cytotoxic and thus patients can fully recover from tetanus toxin poisoning without neuronal damage. We

wanted to express the toxin gene in transgenic mice in order to interfere with synaptic functions. The goals are: (1) to analyze the developmental consequences of impaired synaptic function; and (2) to correlate potential behavioral defects of transgenic mice with the spatio-temporal expression pattern of the transgene.

A synthetic version of the clostridial tetanus toxin light-chain gene was engineered to fit the transcriptional and translational requirements of eucaryots. Experiments in Aplysia neurons showed that RNA made from this synthetic gene is significantly more active than RNA from the original bacterial gene. This gene was also efficiently expressed in mammalian cells.

Several transgenic lines have been established which carried the tetanus toxin gene under the control of either: (1) an SV40 early gene promoter; (2) the promoter of the pro-opiomelanocorticotropin gene (POM-C); or (3) the L7 gene promoter. Increased mortality in some of these lines indicated that the transgene might interfere with important physiological functions. Most strikingly, however, was the finding that all expressing transgenic males, with the notable exception of one founder animal, were sterile regardless of the promoter used. In situ hybridization analysis revealed expression of the transgene in the seminiferous tubule. Histopathological analysis showed the complete absence of mature spermatozoa and indicated that spermatogenesis was blocked at a late stage. Several lines of evidence indicate that the function of Sertoli cells is impaired in these transgenic animals: (1) one highly mosaic male founder gave rise to a transgenic line in which subsequently all transgenic males were sterile; (2) Sertoli cells exhibit characteristic vacuoles and alterations in their cytoskeleton; (3) the expression pattern of the transgene is compatible with expression in Sertoli cells.

These results show for the first time that tetanus toxin can effect cells other than neurons. They indicate that Sertoli cells and neurons have a common molecular toxin-

target. Furthermore, our results suggest that calcium-dependent exocytosis may occur in Sertoli cells and play an important role in sperm cell maturation.

Significance to Biomedical Research:

Analysis of the cellular and molecular mechanisms underlying development of the central nervous system aims to understand better how neurons acquire a specific identity and how they establish and maintain synaptic connections. A better understanding of these processes should greatly facilitate our insight not only into the cause and manifestation of many congenital mental disorders but also into the way in which the adult brain works. The unique feature of our brain is the vast number and enormous complexity of neuronal interconnections. Many of these connections are made during embryonic development strictly determined by the genetic program. Others are made in response to environmental stimuli and as the result of learning processes. We utilize transgenic and chimeric mice to study the role of regulatory genes during neurogenesis and also as a means to interfere with the neurogenic program.

Proposed Course of Project:.

All experiments are in progress and will be continued.

1) The analysis of the regulation of the RARß2 gene expression suggests that a transacting factor with a pair-rule-like expression pattern may regulate the RARß2 expression. However, genes analogous to the Drosophila pair rule type genes have not been identified in vertebrates so far. We are now uisng RARß2 promoter sequences to test our hypothesis and to facilitate the cloning of upstream regulatory genes.

2) Our experiments suggest a role for the open reading frames in the 5' untranslated region of the RARß2 gene in the regulation of the tissue specific expression. This

hypothesis will be tested and the physiological relevance of these open reading frames analyzed by mutating this region via homologous recombination. A construct in which all ATGs of these open reading frames have been mutated has been generated and will be microinjected into ES cells. Mutant cell lines will be analyzed by the polymerase chain reaction and used to introduce the mutation into the mouse germ line.

3) We have engineered a tetanus toxin gene for efficient expression in mammalian cells and demonstrated that the expression of this gene in the seminiferous tubule leads to sterility. In order to mimimize the effort required to analyze the toxin expression we are now planning to link the expression of the tetanus toxin gene to that of a lacZ gene. Our future work will focus on the use of this construct to interfere with synaptic function. Two major aspects will be studied: (i) othe impact of the toxin expression on the establishment and maintenance of synaptic connections; (ii) the correlation of behavioral defects with the spatiotemporal expression pattern.

4) We are using embryonic stem cell lines to create mice with mutations in neurologically important genes. A vector for homologous recombination of the encephalin gene has been constructed. Several embryonic stem cell lines have been obtained with this construct and are currently tested for homologous recombination events. We have also constructed a vector for mutagenesis via gene-traps. This vector permits the efficient expression of a neomycin-lacZ fusion gene upon integration into any actively transcribed region. ES cell lines derived with this construct are first analyzed for expression in neuronal cells and subsequently used to generate germ line chimeric mice.

Publications:

Reynolds K, Mezey E, Zimmer A. Activity of the retinoic acid receptor-ß promoter in transgenic mice, Mech Dev 1991;36:15-29.

Zimmer A. Manipulating the genome by homologous recombination in embryonic stem cells, Annu Rev Neurosci 1992;15:115-37.

Zimmer Z, Wang ZQ, Wagner EF, Gruss P. Homologous recombination in embryonic stem cells as a means to generate mice with defined mutations. In: Gottesman ME, Vogel H, eds. Mechanisms of eucaryotic DNA recombination. San Diego: Academic Press, 1992;29-40.

Zimmer A, Zimmer A. Induction of a RARß2-lacZ transgene by retinoic acid reflects the neuromeric organization of the central nervous system, Development 1992;in press.

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

PROJECT NUMBER
Z01 MH 02622-01 LCB

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Structure and functions of signal-transducing G-proteins

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

PI:	John Northup	Special Expert	LCB, NIMH
Others:	Haya Tamir	Visiting Associate	LCB, NIMH
	David Wildman	Guest Researcher	LCB, NIMH
	Jean Labrecque	Guest Researcher	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 STAFF YEARS:	PROFESSIONAL:	OTHER:
3.5		3.5

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.)

Assays of the biochemical functions of G-protein βγ subunits were used to assess the status of these proteins in ACTH-unresponsive mutant Y1 adrenal cortical cells. Analysis of the expression of the β subunit chains with molecular probes identified a modest decrement of the β2 gene product. However, in comparison with wild-type Y1 cells, the mutant clones displayed significantly diminished βγ activity, indicating a mutation of the gene(s) or defect in post-translational modification. We have constructed baculo viral expression vectors and obtained significant levels of expression of G-protein βγ dimers in Sf9 cells. Techniques were devised to purify the recombinant proteins to homogeneity, and their specific biochemical activities were assessed. The unmodified βγ dimer interacts with identical affinity for α subunit, but diminished affinity for receptor. Thus, prenyl modification not only anchors the βγ dimer to the plasma membrane, but also enhances the receptor interaction of the dimer. A novel mechanism for G-protein activation by in situ GTP synthesis catalyzed by nucleoside diphosphate kinase (NDK) was examined. While in vitro GTP formation using GDP-bound ARF protein was found, when we attempted to extend these observations to the signal transducing G-proteins, all effects of the NDK were found to be artifactual. These studies refute the proposed role for the nm-23 gene products as regulators of G-proteins.

Project Description

Production and characterization of recombinant Gβγ dimers:

In a collaboration with the laboratory of Dr. Michael Dennis, NRC Biotechnology Research Institute, we have produced reconstitutively active G-protein βγ dimers in nanomole quantities in insect cells using the baculovirus-Sf9 insect cell expression system. Sf9 cells were infected either singly or in combination with recombinant baculoviruses containing defined G-protein β or γ gene composition. It was possible to express the β_1 and γ_2 gene products independently of each other in this system, as determined using immunological and metabolic labeling techniques. The ability of recombinant β and/or γ polypeptides to function in defined activities assays was assessed for membrane extracts or supernatant fractions of infected Sf9 cells. Extracts of cells expressing β or γ alone were inactive in our assays, whereas those from cells co-infected with both genes display activity. Labeling with [^{3}H]-mevalonolactone showed that the γ subunits of membrane associated βγ dimers incorporated the radiolabel while in the soluble form they were not (thus they were not post translationally modified). The recombinant dimers facilitated GTPγS binding to $G_t\alpha$ in a reconstitution system which included the photoreceptor rhodopsin (with the membrane extract exhibiting a higher affinity), the dimers also facilitated interaction of pertussis toxin with $G_t\alpha$. Thus, prenylation of γ_2 targets the βγ dimer for membrane attachment but was not required for the functional interaction of the dimer with either the receptor or $G_t\alpha$.

Identification of mutations in Gβγ:

Dr. Bernard Schimmer of the University of Toronto has isolated a number of biologically important mutations in cAMP signalling in the Y1 adrenal cortical tumor line. Dr. Jane Mitchell (MRC sponsored postdoctoral fellow in the laboratory at Yale) has examined forskolin-resistant mutants of Y1 adrenocortical cell line that display a markedly decreased responsiveness to ACTH receptor and somewhat diminished response to post-receptor stimulators of adenylycyclase. These cells contain decreased amounts of the α subunits that mediate the cyclase. The quantity and functional activity of the βγ subunits were examined in those cells using our defined biochemical assays. While the abundance of β subunit chains for β1 and β2 gene products was not consistently different between two independently isolated clones, the βγ activity was decreased by 55%-57% compared with the wild type cells. These results suggested that a mutation in either β or γ subunits or a defect in post-translational modification may result in the nearly complete loss of receptor-stimulation.

Expression of G-protein signalling elements during differentiation:

In the course of our investigations of the structure and functions of G-proteins we have developed a number of molecular probes for the components of several signalling pathways. Haya Tamir has collaborated with Dr. Collin Barnstable, Yale University, to investigate the expression of G-protein signalling pathways in developing rat retinas as a simple model of neural embryology. A panel of rabbit polyclonal antisera specific for

purified native protein components of the visual and neuronal signal transduction pathways was characterized for specificity to rat retinal and brain proteins to study their developmental regulation and localization in rat retinas. The visual G-protein transducin and its effector phosphodiesterase were found to be expressed only in post-natal day 9 pups and only in the photoreceptor cells. In younger pups no expression was detected. In adult retinas the immunoreactivity to the above components remained localized in the outer retinal segment. Antibodies recognizing the neural G-protein G_o were localized in the plexiform and ganglion layers. The small molecular weight GTP binding protein G_p was found to be expressed in the synaptic layer. Surprisingly, G_o reactivity was detected in early post-natal photoreceptors, prior to the expression of the G_t but was undetected in mature photoreceptors. These studies indicate that G-protein expression is distinct for cells of the CNS and that the repertoire of G-protein signalling changes during differentiation.

Novel G-protein pathways:

Dr. Richard Kahn of the NCI has been studying a unique GTP-binding protein, ADP-ribosylation factor (ARF) and its relation to cell growth and cancer. With Drs. Kahn and Randazzo, Dr. Northup had obtained preliminary evidence for a novel mechanism for G-protein activation by in situ GTP synthesis catalyzed by nucleoside diphosphate kinase (NDK). Since several other laboratories had obtained evidence suggesting that such regulation might obtain for G-protein pathways, we wished to critically examine this result. While in vitro GTP formation using GDP-bound ARF protein was found, when we attempted to extend these observations to the signal transducing G-proteins, all effects of the NDK were found to be artifactual. These studies refute the proposed role for the nm-23 gene products as regulators of G-proteins.

Significance to Biomedical Research:

Many of the effective agents for treatment of psychiatric disorders either directly or indirectly with neurotransmitter receptors. In recent years the role of G-proteins in neural signalling has expanded to include not only the regulation of intracellular second messenger pathways (cAMP, inositol phosphates, calcium, eichosanoids) but also direct regulation of ligand-gated ion channels (potassium, calcium). All of these pathways are candidates as targets for psychotherapeutic agents. The research efforts described here are aimed at defining the structural bases for G-protein mediation of receptor signalling. Expression of individual genes encoding G-protein subunits, receptors, and effectors allows the systematic dissection of these pathways in simplified cellular systems. Understanding the molecular details of the actions of neurotransmitters may provide insights into the nature of mental illness as well as new avenues for the design of more efficacious therapeutic agents.

Proposed Course:

The success of our collaboration with Dr. Michael Dennis in expressing of $G\beta\gamma$ subunits in Sf9 cells enables a detailed study of the structure and functions of the dimer. We will continue the expand these studies to include the more disparate structures of the

homologous ste4 and ste18 genes from yeast in order to assess which functions of the βγ dimer are conferred by conserved structure and which are unique to individual gene products. We will also expand our biochemical analyses to include potential novel pathways of regulation by the βγ chains. We have obtained preliminary data finding that a phospholipase C is activated by βγ as well as αq and we plan to determine the βγ specificity of this. We will also examine the β-adrenergic and muscarinic receptor kinase which has recently been found to be stimulated by βγ. Use of the recombinant βγ products will allow facile examination of the important structural features of the molecules by construction of chimeras and site-directed mutagenesis of the β and γ chains.

The in vitro examination of the specificity of G-protein interactions has been limited to date by the lack of adequate systems of receptor-effector depleted in specific G-protein subunits. We will extend the techniques we have devised for the in situ uncoupling and recoupling of rhodopsins to other receptors. In collaboration with Dr. Michael Dennis, we have already examined Sf9 cells infected with baculoviruses containing M2 muscarinic and 5HT1A receptors for suitability. Further experimentation will determine optimal procedures for in situ depletion of G-protein subunits and efficacious reconstitution. Such systems will allow us to test hypotheses concerning the individual roles of α,β, and γ subunit chains in receptor recognition. We will collaborate with units in the LCB to express and reconstitute G-protein interaction with other receptor-homologous DNAs which have been cloned. This may provide an additional strategy for the identification of the functions and ligand specificities of "orphan" receptors.

We plan to expand our examination of the cellular expression of signal transduction elements to regions of the CNS in addition to the retina. We will examine the regional distribution of β and γ gene expression in comparison with α and identified receptors to determine if individual β and/or γ genes are affiliated uniquely with specific receptor pathways. In an alternative approach to this question, we will screen neurally-derived clonal cell lines known to express differentiated signalling pathways for mRNAs of β, γ and α chains to identify unique associations. Suitable clones will be chosen for disruption of β, γ, and α subunit expression by antisense constructs.

At this time the only information concerning the structure of the molecules involved in G-protein signalling are the amino acid sequences deduced from cDNA clones. While a crystal-structure has been determined for the hydrophilic portion of the ras proto-oncogene and a structure has been determined for a rhodopsin in fused-membranes of a halobacter, none of the signalling components identified to function in the nervous system have yet been crystallized. We plan to use our recombinant expression systems to produce homogenous gene-products in sufficient abundance to crystallize for X-ray diffraction studies. Alternatively, we will examine the suitability of photoreceptor-derived proteins for this purpose. Crystallization of these molecules presents a formidable challenge, as techniques for efficient and reproducible crystallization of membrane proteins have not been devised. This project will thus be limited by the ability to produce substantial quantities of scrupulously homogeneous individual molecular species to pilot novel crystallization methods. It is hoped that such techniques might subsequently be applied to related molecular species available in lesser quantities, but it is likely to be accomplished only for

the model systems which we initially choose. Nevertheless, the information derived from such studies will be of paramount significance in identifying the actual molecular structures involved in G-protein signalling.

The biochemical methods and molecular reagents used in these studies will be available to the LCB and generally to the DIRP NIMH to investigate receptor signalling pathways. We hope thereby to identify those pathways critical to CNS functioning and the pathophysiology of mental diseases.

Publications:

Randazzo, PA, Northup, JK, and Kahn, RA. Activation of a small GTP binding protein (ARF) by nucleoside diphosphate kinase catalyzed phosphorylation of bound GDP, Science 1991; **254:** 850-3.

Randazzo, PA, Northup, JK, and Kahn, RA. Regulatory GTP binding proteins (ARF, Gt, and RAS) are not activated directly by nucleoside diphosphate kinase, J Biol Chem 1992; in press.

Mitchell, J, Northup, JK, and Schimmer, BP. Defective guanyl nucleotide-binding protein $\beta\gamma$ subunits in a forskolin-resistant mutant of the Y1 adrenocortical cell line, Proc Natl Acad Sci USA 1992; in press.

Wildman, D E, Tamir, H, Leberer, E, Northup, JK. and Dennis, M Prenyl modification of guanine nucleotide regulatory protein $\gamma 2$ subunit is not required for interaction with transducin α subunit or rhodopsin, Proc Natl Acad Sci USA 1992; in press.

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

PROJECT NUMBER
Z01 MH 00881-36 LCM

PERIOD COVERED
October 1, 1991 to September 30, 1992

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,

PI:	E.E. Kaufman	Research Chemist	LCM, NIMH
Others:	T. Nelson	Medical Officer (Research)	LCM, NIMH
	B.F. Driscoll	Research Biologist	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 STAFF YEARS:	PROFESSIONAL:	OTHER:
0.85	.6	0.25

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

Summary
The neuroactive compound, γ-hydroxybutyrate (GHB), has been found to inhibit C-AMP formation in homogenates from rat brain. To determine in which cell type this effect occurs we have studied the influence of GHB on C-AMP formation in both neurons and astroglial cells in primary culture. In neurons the effect of GHB is to inhibit C-AMP formation whereas in the astroglial cells there is either no effect on stimulation of C-AMP formation. The effect of GHB on C-AMP formation reinforces the simularity between GHB and the opioid peptides.

Work on the degradative pathway for GHB has continued. We are now able to estimate the contribution of the two enzymes, the cytosalic GHB-dehydrogenase and the mitocondrial hydroxyacid-oxoacid transhydrogenase, to the initial rate-limiting step in this pathway. Preparation of an antibody to the to the transhydrogenase will enable us to carry out precise localization of this new metochondrial enzyme.

Project Description:

Objectives

Purification and characterization of the mitochondrial hydroxyacid-oxoacid transhydrogenase will continue to be an objective of this project. The purified enzyme will be used for determination of a putative bound cofactor and additional kinetic studies.

The similarity between the physiological effects of γ-hydroxybutyrate and the opiates have suggested that γ-hydroxybutyrate, like the opiates, may exert its effect by decreasing the intracellular concentration of C-AMP. The objective of this part of the project is to determine which of the 2nd messenger systems are affected by γ-hydroxybutyrate, whether they are inhibited or stimulated by this compound and finally how their effect is mediated.

Methods

Differential centrifugation, ammonium sulfate fractionation, and chromatography on DEAE cellulose, sephadex G-150 and substituted sepharoses have been used in the purification of the transhydrogenase.

The ^{125}I Scintillation proximity assay has been used for the measurement of tissue levels of C-AMP. A similar scintillation proximity assay will be used to measure tissue levels of C-GMP. Ca^{++} uptake into both neurons and astrocytes as well as efflux from these cells is being studied with ^{45}Ca. Fluorescent Ca^{++} chelating indicators such as Fura-2. amd Fura-3 will be used to examine the effect of γ-hydroxybutyrate on intracellular Ca^{++} concentrations.

Findings

The partially purified enzyme has been used for kinetic studies and for inhibitor studies. The enzyme has been shown to exhibit ping-pong kinetics. D-β-hydroxybutyrate and L-malate have both been found to be inhibitors of this enzyme.

Preliminary studies indicate that in the range of 10^{-4}-10^{-6}M γ-hydroxybutyrate can significantly inhibit C-AMP formation in homogenates of both hypothalmus and hippocampus. GHB has been found to have an effect on C-AMP formation in primary cultures of both neurons and astroglia. Early results from the Ca^{++} uptake studies show that 5 x 10^{-6}M γ-hydroxybutyrate (twice the normal mean concentration found in whole brain), can inhibit Ca^{++} uptake in unstimulated neurons in culture by as much as 50%.

Significance to Biomedical Research

It has been established that there is a dual pathway for the initial rate-limiting step in the catabolism of γ-hydroxybutyrate. One arm of this pathway is catalyzed by γ-hydroxybutyrate dehydrogenase, a cytosolic $NADP^+$-dependent oxido reductase and the other by the mitochondrial hydroxyacid-oxoacid transhydrogenase.

Characterization of these enzymes will enhance our understanding of how this neuroactive compound is disposed of and of factors which regulate tissue levels of γ-hydroxybutyrate.

γ-Hydroxybutyrate has been known for some time to produce numerous and sometimes striking physiological changes when administered to animals in pharmacological cases. Many of these physiological changes are similar to those produced by the opiates. More recently it has been found to have marked protective effects against tissue damage in forebrain neuronal ischemia in the rat and against tissue damage caused by regional intestinal ischemia in the hamster. We are now initiating experiments on the effect of γ-hydroxybutyrate on several 2nd messenger systems which we anticipate will increase our understanding of how γ-hydroxybutyrate exerts the numerous physiological and protective effects observed in experiments on laboratory animals as well as the beneficial effects observed in clinical trials on the treatment of narcolepsy and alcohol withdrawal syndrome.

Proposed Course

Work on the purification of the hydroxyacid-oxoacid transhydrogenase will continue. The purified enzyme will be used for 1)further kinetic studies, 2)identification of the putative bound cofactor, 3)for antibody production and 4)to clone the gene for this enzyme.

Work has been initiated on the effect of γ-hydroxybutyrate at the cellular level. We plan to investigate the effect of γ-hydroxybutyrate on C-AMP, C-GMP and calcium flux. How γ-hydroxybutyrate exerts its effect on 2nd messenger systems will then be examined.

Publications

Kaufman, EE and Nelson, T. An overview of γ-Hydroxybutyrate catabolism: The role of the cytosolic $NADP^+$-dependent oxido-reductase EC 1.1.1.19 and of a mitochondrial hydroxyacid-oxoacid transhydrogenase in the initial, rate-limiting step in this pathway. Neurochemical Research 1991; 16: 965-974.
MH04214-05

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

PROJECT NUMBER
Z01 MH 00882-25 LCM

PERIOD COVERED
October 1, 1991 to September 30, 1992

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,

PI:	L. Sokoloff	Chief	LCM, NIMH
Others:	C. Kennedy	Medical Officer (Research)	LCM, NIMH
	M. Lyon	Guest Researcher	LCM, NIMH
	G.A. Dienel	Chemist	LCM, NIMH
	C.B. Smith	Research Chemist	LCM, NIMH
	T. Nelson	Medical Officer (Research)	LCM, NIMH
	K. Adachi	Visiting Fellow	LCM, NIMH

COOPERATING UNITS (if any)
Laboratory of Neuropsychology, NIMH (M. Mishkin & M. Webster); Stroke Branch, NINDS, (J.M. Hallenbeck & K.U. Frerichs)

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.5	6.5	2.0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

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

SUMMARY:

This project is directed toward the development, validation, and application of methods for measurement of physiological and biochemical processes in functional and structural regions of the nervous system. The methods are first designed for use in animals with quantitative autoradiography and then adapted for use in man with PET. The [^{14}C]iodoantipyrine ([^{14}C]IAP) method for measuring local cerebral blood flow (lCBF) and the [^{14}C]deoxyglucose ([^{14}C]DG) method for measuring local cerebral glucose utilization ($lCMR_{glc}$) were developed in this program; adaptations of these methods are the only generally accepted quantitative methods routinely used in man with PET. These methods are applied to various physiological, pharmacological, and pathological states. Efforts this past year have been directed toward these goals: 1) evaluation of necessary parameters to apply the DG method to pathological states when the values of these parameters may deviate from normal; 2) development of a sequential double label procedure to study control and altered behavioral states in the same animal with the autoradiographic methods; 3) model development and evaluation to improve accuracy and reliability of the [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) adaptation of the [^{14}C]DG method for human use with PET.

Other Invesstigators (continued):

K. Schmidt	Computer Systems Analyst	LCM, NIMH
N. Cruz	Biologist	LCM, NIMH
A. Crane Tannenbaum	Biologist	LCM, NIMH
S. Takahashi	Visiting Fellow	LCM, NIMH
A. Breier	Guest Researcher	LCM, NIMH
E. Palombo Kinne	Contractor	LCM, NIMH
F. Orzi	Contractor	LCM, NIMH

PROJECT DESCRIPTION:

Studies this year include: 1) applications of the [^{14}C]DG method in experimental physiological, pharmacological, and pathophysiological states; 2) methodological studies to extend the DG method to pathological states, to other metabolic pathways and systems, and to the improvement of the accuracy of its [^{18}F]fluorodeoxyglucose modification for use with PET; and 3) experiments to adapt the DG method for sequential ^{3}H/^{14}C double label quantitative autoradiographic measurements of $lCMR_{glc}$, thus allowing two successive determinations and comparisons of two behavioral states (e.g., control and experimental) in the same animal within a time frame of 50-60 minutes. The individual studies will be described separately.

I. APPLICATIONS OF THE DEOXYGLUCOSE METHOD

A. MPTP-Induced Parkinsonism in Primates. Previous studies in collaboration with L. Porrino, I. Kopin, and others in the NINDS delineated by metabolic mapping with [^{14}C]DG the constellation of local cerebral effects produced by MPTP-induced bilateral and unilateral Parkinsonism in monkeys. The results of the studies of the acute and chronic effects of MPTP have been published in a series of papers over the last several years. In these studies it was found that there is a progression of changes in the clinical picture of the parkinsonian syndrome with time following the administration of the neurotoxin, and the information about the local cerebral metabolic changes resided in the autoradiograms derived from our previous experiments. Dr. E. Palombo Kinne, who was previously a Visiting Fellow in our Laboratory and worked on this project, returned for a couple of months to analyze the autoradiograms and complete the computations of $lCMR_{glc}$. A manuscript on the results of these studies, which relates the time course of the local metabolic changes to those of the clinical signs is currently in the stage of final revision.

B. Cerebral Cortical Activity Accompanying the Performance of a Visually Cued Task. The continuing analyses of data obtained a few years ago in monkeys engaged in a motor task induced by visual cues has elucidated further the distribution of changes in functional and metabolic activities in the brain under these special conditions. Monkeys were trained by the Laboratory of Neuropsychology, NIMH, to discriminate illuminated patterns on a screen and to press a particular panel with one hand in order to receive a water reward while their local cerebral metabolic rates were being determined with the [^{14}C]DG method. These experiments were carried out in intact control monkeys and in another group of

monkeys in which the hemisphere contralateral to the moving hand had been surgically deprived of all visual input by sectioning of one optic tract, the corpus callosum, and the forebrain commissure several weeks previously. The animals were then retrained following recovery from the surgery to perform their task as previously. The results obtained in the [^{14}C]DG studies in these two groups of animals led to the identification of all areas of the cerebral cortex, striate and beyond, that respond to visual input (Macko, K.A., et al., Science **218**:394, 1982). Recent additional analyses of the results in both the intact and experimental animals revealed unilateral metabolic effects of the visually induced motor task in the motor and sensory cortical areas and in the parietal lobe (areas 5 and 7) contralateral to the moving hand. This was found in both groups of animals; in the case of the surgically altered animals these effects were in the "blind" hemisphere. These results have been further examined in great detail by H. Savaki and C. Kennedy to address at least two additional questions: (1) the distribution of altered metabolic activities in the various parts of the motor pathway when an animal engages in the panel-pressing task; (2) an explanation of how a hemisphere deprived of visual input is able to initiate and sustain activity in the motor pathways of that same hemisphere in response to visual cues. Portions of the results were presented at and published in the proceedings of a Benzon Symposium in Copenhagen, Denmark, August, 1990. A more complete manuscript on this work is in press in the *Journal of Neuroscience*.

C. Effects of Stress Induced by Blockade of Glucose Utilization with Pharmacological Doses of 2-Deoxyglucose on Local Cerebral Blood Flow. Walter Cannon had included hypoglycemia as one of the conditions of stress that activated the sympathetic system, and insulin-induced hypoglycemia has been used to treat schizophrenia, though with doubtful success. It is generally recognized, however, that schizophrenic patients are less responsive to both insulin-induced hypoglycemia or epinephrine-induced hyperglycemia. A. Breier has proposed the use of stress induced by pharmacological doses of DG as a test for the responses of schizophrenia to stress if this test could be combined with measurements of a physiological function relevant to stress that could be assayed with PET. It is not possible to measure $lCMR_{glc}$ in the presence of pharmacological doses of DG, but lCBF can readily be measured, and it is in generally well correlated with $lCMR_{glc}$. A. Breier and A. Crane Tannenbaum, therefore, examined the effects of loading doses of DG, short of those that produce coma, on lCBF in rats measured with our [^{14}C]iodoantipyrine technique. They found marked increases in blood flow, approximately 100% increases, generally distributed through all structures of the brain. These results indicate that the measurement of lCBF in man with PET can be useful to gauge responses to stress and may be useful to compare stress responses of normal and schizophrenic subjects. These results are to be presented to the annual meeting of the Society of Neuroscience in Anaheim, CA., in November, 1992.

D. Metabolic Development of the Primate Visual System. This is an extension of earlier published work on the delineation of the regions of monkey cortex anterior to the striate cortex which become activated by a patterned visual stimulus. These are studies done in collaboration with M. Webster and M. Mishkin of the Laboratory of Neuropsychology, NIMH. It was previously noted in studies with the [^{14}C]DG method that very few regions

other than the primary visual cortex could be metabolically activated in the newborn. By conducting studies at various postnatal ages with the DG method the functional maturation of visually responsive regions is being determined. Personnel changes and limitations in available trained personnel as well as monkeys allows only slow progress in this study. This year a few additional experiments were conducted, and the data are presently being analyzed.

E. Effect of Electrical Stimulation of Fastigial Nucleus on Local Cerebral Blood Flow in the Rat. It is generally believed that there is little if any neurogenic regulation of cerebral blood flow (CBF). The dural, pial, and intracerebral blood vessels are innervated by myelinated and unmyelinated fibers, and when stimulated these nerves may cause either constriction or dilatation of the vessels, but the evidence that these pathways have any significant role in the regulation of brain perfusion is unimpressive at best. Nevertheless, adherents of the view that neurogenic control of the cerebral circulation regularly come to the fore with so-called demonstrations to support their view. The latest is a series of reports by C. Iadecola and others that claim large global increases in CBF as a result of stimulation of the fastigial nucleus of the cerebellum in rats. If true, this is a very important finding. On critically examining these published reports, however, we find a possible combination of circumstances resulting from the altered physiological conditions of their animals (e.g., paralyzed, artificially ventilated, "hemorrhaged") which could have led to artifactual results. We are addressing this important question in awake and unanesthetized rats under normal physiological conditions by using our [^{14}C]iodoantipyrine method. The studies were initiated by F. Orzi and A. Tannenbaum and are being continued by S. Takahashi. Thus far, we find no evidence that stimulation of the fastigial nucleus results in increased cerebral blood flow in the unanesthetized rat.

F. Effects of Hibernation on Local Cerebral Blood Flow and Glucose Utilization. John Hallenbeck, NINDS, has initiated studies of hibernation in the ground squirrel to determine the mechanisms that protect the brain from the almost arrested cardiac and circulatory functions that occur in the hibernating state. Such knowledge could be useful in the design of procedures in the management of cerebrovascular disease, particularly stroke. A joint project has been undertaken to measure cerebral blood flow (CBF) and cerebral glucose utilization (CMR_{glc}) in hibernating squirrels. The results thus far indicate that average blood flow to the brain as a whole is profoundly reduced in hibernation, far below the levels required to sustain consciousness in non-hibernating animals. This profound reduction occurs without altered arterial blood gases, and the mechanism of the reduction is unknown. It is likely to be secondary to reduced energy metabolism, and experiments to measure local CMR_{glc} by means of the deoxyglucose method have been initiated.

G. Comparison of Local Rates of Cerebral Blood Flow in Partially Restrained and Unrestrained Rats. Measurements of cerebral blood flow or glucose utilization in rats are usually made in partially restrained animals. This may be disadvantageous in experiments in which even mild stress might affect the results. A. Tannenbaum has found that the Sprague-Dawley rat can be trained to remain relatively immobile in a triangular black paper

tent without any form of restraint. To determine whether the usual preparation of rat, which involves partial restraint of the lower torso and hind limbs, alters their physiologic state, she is comparing rats under the unrestrained and restrained conditions by measuring a number of physiological variables e.g. plasma corticosterone, arterial pressure, blood gas tensions, local cerebral glucose utilization and blood flow. The study may provide new information on the effects of mild "stress" (resulting from restraint) on these functions.

II. METHODOLOGICAL STUDIES

A. Effects of Tissue Heterogeneity on Determinations of Local Cerebral Blood flow and Glucose Utilization. The first explicitly stated assumption of both the iodoantipyrine and deoxyglucose methods is that the tissues to which they are applied are homogeneous with respect to the physiological or biochemical processes being examined and to the concentrations of the relevant labeled compounds in the tissue. This assumption is reasonably well approximated with quantitative autoradiography which has a spatial resolution of about 200 μm. With PET scanning, however, the spatial resolution is 1 cm or more, and because of partial volume effects homogeneous tissues are rarely, if ever, achieved. Unfortunately, the assumption of homogeneity is frequently forgotten or ignored, and serious misinterpretations and misuses of the methods have resulted. Because of the extensive work done in this portion of the overall project, it is being reported in a separate individual project report prepared by K. Schmidt as the Principal Investigator (See Z01 MH 02569-02).

B. Adaptation of the Deoxyglucose Method for Use in Focal Pathological Conditions. Many pathophysiological conditions, such as focal seizures, ischemia, tumors, etc. involve changes in functional activity and metabolic rate that are confined to specific areas of the brain. To apply the DG method for measurement of local rates of glucose utilization in these specific regions with the focal abnormalities determination of the local lumped constant of the DG method simultaneously with the measurement of local glucose utilization in the same region is required. Because the value for the lumped constant varies only with tissue and glucose content, the lumped constant can be determined from the relationship between tissue glucose content and the lumped constant that was directly experimentally determined in this Laboratory by G. Dienel, N. Cruz, and K. Mori in previous years. The local brain glucose contents can be determined from the steady-state brain tissue:plasma distribution ratio for 3-O-methylglucose which distributes between brain and plasma in a quantitative relationship the glucose tissue:plasma distribution ratio. Our previous, recently-published studies established the steady-state relationships among the distribution spaces for glucose, deoxyglucose, and methylglucose and the lumped constant in brains of rats in which the brain glucose content was controlled by regulation of the plasma glucose level, i.e., the glucose supply to the brain. On theoretical grounds, however, the relationship between the methylglucose distribution ratio and the tissue glucose content, and, therefore, also the lumped constant, should be different when the brain glucose content is altered by the rate of tissue glucose utilization (e.g., by very high or very low rates of glucose utilization during

seizures or metabolic depression, respectively) rather than by glucose supply. The influence of variations in cerebral glucose utilization the relationship between brain glucose content and methylglucose distribution ratio is, therefore, now being experimentally examined in this Laboratory. Focal changes in metabolic rates are induced by topical application of drugs to the surface of the brain that stimulate or depress local rates of glucose utilization (e.g., penicillin and barbital). The results of experiments by Dienel, Cruz, H. Nakanishi, and K. Adachi demonstrated that changes in the rate of glucose utilization in the brain does alter significantly the relationship between brain glucose content and methylglucose distribution ratio, but the lumped constant remains relatively stable even when the cerebral metabolic rate varies from 50-200% of normal. Further studies of the quantitative relationships among rate of glucose utilization, tissue glucose content, and methylglucose distribution space are still in progress but almost completed. When these relationships are established, we will have all of the necessary information for using methylglucose to determine local glucose contents and, therefore, also local lumped constants simultaneously with the determination of local glucose utilization. The DG method will then be fully applicable to focal pathophysiological conditions.

Methylglucose was used by Cruz and Dienel to demonstrate regional stability of brain glucose levels with time after portacaval shunting. Portacaval-shunted rats are used as an animal model for chronic liver disease. $lCMR_{glc}$ in many structures of non-fasted portacaval-shunted rats was previously shown by Cruz and Duffy to be depressed during the first 4 weeks after portacaval shunting, and to rise progressively to normal or higher than normal values from 4-12 weeks. The question remained whether these local increases in $lCMR_{glc}$ were secondary to changes in tissue glucose levels. Global brain glucose levels and local brain:plasma distribution ratios for methylglucose were shown to be constant between 1 and 12 weeks after shunting, indicating that the local lumped constant would not have changed during the time when there were local increases in $lCMR_{glc}$. The selective, time-dependent changes in $lCMR_{glc}$ after portacaval shunting therefore reflect local, probably functional, responses to chronic hyperammonemia and inadequate liver function. The results of this study were presented at the annual meeting of the American Society for Neurochemistry in Houston, March, 1992.

There still remains one problem to be solved to use labeled methylglucose to determine the local lumped constants needed to adapt the deoxyglucose method to focal pathophysiological conditions *in vivo*. It is necessary to perfect the procedure for accurate, quantitative double label $^{3}H/^{14}C$ autoradiography. Studies to achieve this have been in progress for sometime by Drs. C. Smith, H. Nakanishi, B. Agranoff, and C. Kennedy. A major obstacle is the elimination of errors due to differential self-absorption of the low energy β-radiation of ^{3}H in the various cerebral structures. Presumably this is due primarily to the different contents of lipids in the various brain regions. The approach has been to extract the lipids from the brain sections at 4°C with anhydrous hexane prior to their autoradiography. This has been shown to reduce or eliminate the differential self-absorption, everywhere except in the cerebellum, without washing out intolerable amounts of the water-soluble labeled compounds. A second problem has been the

separation of the individual contributions of ^{3}H and ^{14}C to the optical densities in the autoradiograms. This problem has been solved by the use of mylar film, 6-12 μm in thickness, to absorb all the radiation of ^{3}H with only slight attenuation of the ^{14}C radiation for the ^{14}C autoradiograms and the use of 200-400 fold more ^{3}H than ^{14}C to minimize the contribution of the ^{14}C to the ^{3}H autoradiograms; the residual contribution of the ^{14}C can be accounted for by our computerized image-processing systems. Therefore, images obtained from two separate exposures of the same sections together with calibrated standards, one with and one without mylar film, when subtracted one from the other with the aid of an image-processing system, provide the local concentrations individually of both labeled glucose analogues. We are in the process of calibrating the ^{3}H- and ^{14}C-labeled methymethacrylate autoradiographic standards for their equivalent concentrations in brain sections 20μm thick. When this is completed the local distribution spaces for methylglucose and, therefore, also the local lumped constants and the local rates of glucose utilization in brain will be determined in the same animal in various pathophysiologic states.

C. Use of DG for Measurement of Glucose Utilization in Isolated Cultured Neurons and Astrocytes *in vitro*. Studies by B. Driscoll, G. Dienel, and N. Cruz to examine the tissue:medium distribution ratios for glucose and DG in cultured neurons and astrocytes are also in progress. The aim of this work is to expand the model of the DG method to measure glucose metabolism to include intracellular and extracellular compartments. Preliminary results indicate that cultured astrocytes appear to have a pool of free glucose that is inaccessible to hexokinase; cultured neurons either do not have this pool, or the pool is much smaller than that in astrocytes.

D. Use of DG for Assessment of Other Metabolic Pathways. In the course of the above studies Dienel and Cruz found that DG-6-P was further metabolized in brain to acid-labile compounds. Because DG-6-P is generally thought to be the major metabolite of DG, knowledge of its further metabolism could permit its use for quantitative measurement of the rates of flux in additional metabolic pathways other than glycolysis in brain *in vivo*. The major metabolites of deoxyglucose in plasma and brain were, therefore, separated by HPLC and identified, and their concentrations at different plasma glucose levels determined. Based on these preliminary results Dienel and Cruz are currently examining several areas in which DG might be used to measure rates of other metabolic processes:

1) Local Pentose-Phosphate Shunt Activity. Assay of pentose phosphate shunt activity *in vivo* is of interest because shunt activity in adult brain is believed to be coupled to rates of oxidation of catecholamines by monoamine oxidase. The shunt pathway also provides NADPH for removal of peroxide by glutathione peroxidase, thereby protecting the tissue against oxidative damage. 6-Phosphodeoxygluconate was found in brain, indicating that DG does enter the shunt pathway, and determination of local pentose shunt activity with [1-^{14}C]DG and [6-^{14}C]DG might be possible; after conversion to a pentose ^{14}C would be lost from [1-^{14}C]DG but not from [6-^{14}C]DG, and shunt activity could then be calculated as the difference in rates of glucose utilization determined with [6-^{14}C]DG minus that with [1-^{14}C]DG. Dienel, Cruz, and others, therefore,

used [1-^{14}C]DG and [6-^{14}C]DG to assess shunt activity but found the rate of decarboxylation of 6-phosphodeoxygluconate to be too low for this approach to be feasible. A report of these studies is currently in press in the *Journal of Neurochemistry*.

2) Incorporation of DG-1-P into oligosaccharides. The synthesis and turnover of glycoproteins and oligosaccharides are difficult to assay *in vivo* due to incorporation of label from precursors into other compounds. Because the distribution of label from metabolism of labeled DG is limited, and because one of its metabolites, DG-1-P, is an analog of both glucose-1-P and mannose-1-P, which are precursors of UDP-glucose and GDP-mannose, DG-1-P may be useful to assay oligosaccharide turnover and incorporation into glycoproteins. Recent experiments showed that DG-1-P, an acid-labile compound, is rapidly synthesized by the phosphoglucomutase reaction and accounts for about 7% of the DG metabolites in brain 45 minutes after a pulse of DG; its incorporation into glycoproteins is being examined.

3) Function of Glucose-1,6-P_2. Glucose-1,6-P_2 is thought to have still unidentified regulatory functions in brain. Current experiments have shown that its analog, DG-1,6-P_2, a prominent acid-labile metabolite of DG which was never previously detected in brain, is synthesized in significant amounts (20-30% of the DG-6-P content in brain 45 minutes after a pulse of DG) and is much more sensitive to energy failure than the other metabolites of DG. DG-1,6-P_2 may be useful to assess turnover and putative regulatory functions of glucose-1,6-P_2.

III. Sequential Double Label Deoxyglucose Measurements. With true quantitative double label ^{3}H/^{14}C autoradiography available, it will become possible to complete the development of an accurate sequential double label procedure with which to do two consecutive deoxyglucose measurements in the same animal in two different behavioral states. Work toward this goal was done previously mainly by H. Nakanishi, C. Kennedy, and C. B. Smith in collaboration with B. Agranoff. Experiments were carried out with monkeys studied with one eye patched in the first measurement and the other eye patched in the second period to image separately the ocular dominance columns for the two eyes. One problem is that there is still sufficient residual label left in the plasma from the first deoxyglucose injection to be reflected in the second altered behavioral state. M. Lyon has done experiments in rats in which he has given an injection in a normal state and then at the time that the second behavioral state would normally begin and the second sequence begun, he initiated electrical stimulation of the sciatic cord and found that the initial label reflected the effects of the stimulation. Exchange transfusions to wash out the first label before beginning the second sequence have been tested to minimize this problem, but the results, though encouraging, have not yet resolved the problem. This work is in press in *Neuroscience Letters*.

Significance to Biomedical Research:

It is the pioneering work done in this project that established the foundation of the field of functional brain imaging with PET. It is also the work of this Section that sets the standards in the field. In addition, the applications of its own methods in animals and man has provided much of the knowledge of the physiology and pharmacology of the cerebral circulation and energy metabolism and their relationships to functional activity in the nervous system. The work done in this project also provided neurobiologists with the currently powerful and widely used tool, metabolic mapping, to map functional neural pathways and to identify regions of the brain with altered functional activity in normal, physiologically or pharmacologically altered, or disease-altered behavioral states.

PROPOSED COURSE:

The development of the double label autoradiographic method for simultaneous determinations of local lumped constants and local rates of glucose utilization will continue to be pursued. When completed, it will be tested and, if successfully validated, it will be made operational in studies of conditions with pathophysiological foci within the brain, e.g., ischemia, hypoxia, hypoglycemia, focal seizures, tumors, etc.

Studies will be continued both *in vivo* and in cell cultures of neurons and astroglia *in vitro* to expand the model of the standard deoxyglucose method to include intracellular and extracellular compartments. This expansion should be important to the interpretation of results obtained in some pathophysiological conditions, such as hypoglycemia as well as to define the still unknown comparative properties of neurons and astroglia in their handling of carbohydrates for energy metabolism.

Efforts will be continued to perfect the sequential double label deoxyglucose technique so that it can be applied to studies of learning and memory in the primate in collaboration with B. Agranoff of the University of Michigan and Dr. Mishkin of the Laboratory of Neuropsychology, NIMH.

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Lyon, M. J., Agranoff, B. W., Sokoloff, L., Smith C. Beebe: Residual effects of tracer in sequential double label deoxyglucose studies. *Neuroscience Letters*. (in press).

Dienel, G. A., Cruz, N. F., and Sokoloff, L.: Metabolites of 2-[^{14}C]deoxyglucose in plasma and brain: Influence on rate of glucose utilization determined with deoxyglucose method in rat brain. *J. Cereb. Blood Flow Metab*. (In press).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 00889-13 LCM

PERIOD COVERED
October 1, 1991 to September 30, 1992

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,*

PI:	C. B. Smith	Research Chemist	LCM, NIMH
Others:	L. Sokoloff	Chief	LCM, NIMH
	G. Deibler	Research Chemist	LCM, NIMH
	Y. Sun	Visiting Fellow	LCM, NIMH

COOPERATING UNITS *(if any)*
Dept. Neurosurgery, U. Texas (M. Kadekaro); Dept. of Neurology, U. of Rome (F.Orzi)

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	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.)*

A quantitative autoradiographic method for the determination of local rates of protein synthesis in brain in vivo ($lCMR_{leu}$) has been developed with L-[1-^{14}C]leucine as the tracer amino acid. A four- -compartment model for the behavior of leucine in brain has been analyzed, and an equation has been derived that defines the rates of leucine incorporation into protein in terms of the time course of plasma leucine specific activity, final tissue concentrations of ^{14}C, and λ_i, the steady state ratio of the distribution volumes for the labeled and unlabeled leucine in the immediate precursor pool for protein synthesis. λ_i is a measure of the fraction of leucine in the precursor pool for protein synthesis that is derived from plasma; the remainder is derived from protein degradation. The value of λ_i for the brain as a whole in conscious, adult, male rats has been experimentally determined to be 0.58. λ_i does show some regional variation but all brain regions examined were within 14% of the whole brain value. λ_i increases under light thiopental anesthesia, is unchanged with repetitive electrical stimulation and decreases in regenerating cranial nerve nuclei. These results show that, in general, the amino acid precursor pool for protein synthesis is partially derived from protein degradation. The fraction of the pool coming from protein degradation is not uniform in all brain regions and changes with under some conditions. $lCMR_{leu}$ is decreased under barbiturate anesthesia, increased in regenerating cranial nerve nuclei and unchanged during electrical stimulation. Studies carried out on applications of the method that examine some basic neurobiological questions include: 1) barbiturate & ketamine anesthesia; 2) electrical stimulation; 3) regeneration and 4) chronic cocaine administration.

Project Description:

Major Objectives:

The overall objectives of this research project are:
1. Develop and validate a method for measurement of local rates of protein synthesis in brain.
2. Apply the method to the study of neurobiological problems.

The specific aims pursued during this fiscal year are:
1. Effects of electrical stimulation of the sciatic nerve on λ_i and $lCMR_{leu}$.
2. Time course of changes in λ_i in a regenerating cranial nerve nucleus following axotomy.
3. Effects of anesthetic agents on 8,23 λ_i and $lCMR_{leu}$.
4. Effects of chronic cocaine administration on $lCMR_{leu}$ in rats.
5. Effects of "flooding" with L-valine on $lCMR_{val}$ with the quantitative autoradiographic method.

Methods Employed:

1. Determination of λ_i. λ_i is the ratio of the steady state distribution ratios for labeled and unlabeled leucine between the tRNA-bound pool in the tissue and arterial plasma. Ψ_i is the equivalent ratio for the acid-soluble tissue pool. Because leucyl-tRNA concentrations are too low to determine λ_i in individual brain regions, λ_i is calculated from determined values of Ψ_i and the linear relationship between Ψ_{WB} and λ_{WB}. A pure tRNA-bound and /or an acid-soluble amino acid fraction are extracted from brain after rats are maintained for 60-90 min in a steady state for labeled leucine with a programmed infusion. The specific activities of these fractions from brain and the acid-soluble fraction from arterial plasma are determined and values of λ_i and Ψ_i are calculated.
2. Determination of local rates of cerebral protein synthesis. Animals are administered i.v. L-[1-^{14}C]leucine (100 μCi/kg), timed arterial blood samples are drawn for determination of the time course of plasma leucine specific activity. At 60 min the animal is killed, free [^{14}C]leucine in tissue sections is removed by fixation and washing in 4% formalin and concentrations of [^{14}C]protein in brain regions are determined by quantitative autoradiography. These values, the calculated integrated specific activity of [^{14}C]leucine in the tissue, and λ_i are used in the operational equation of the method to calculate $lCMR_{leu}$. L- [1-^{14}C]valine is used as the tracer in some experiments; the procedures are identical to those used in the [^{14}C]leucine experiments except that the experiment interval is 90 min. The values of λ_{WB} for valine under control and "flooding" conditions are 0.37 and 0.74, respectively.

Major Findings:

1. Effects of electrical stimulation on $lCMR_{leu}$. The effects of repetitive, unilateral electrical stimulation of the sciatic nerve at 10 Hz on the value of Ψ_i in dorsal root ganglia (L4-L6) and dorsal and ventral horns of lumbar spinal cord were evaluated by side-to side comparisons in nine rats. No differences in Ψ_i between control and stimulated sides were found.

Values of λ_i calculated from the values of Ψ_i were used in the determination of $lCMR_{leu}$ under these conditions of stimulation. No effects of stimulation on $lCMR_{leu}$ were found in any of the regions examined. These results were reported at the 1992 meeting of the American Society for Neurochemistry in Houston.

2. Evaluation of λ_i in the regenerating hypoglossal nucleus. We have determined the time course of the effects of unilateral hypoglossal axotomy on the value of Ψ_i in regenerating hypoglossal nucleus. These results are used to calculate values of λ_i for the determination of $lCMR_{leu}$ under these conditions. Values of Ψ_i were unchanged at Post-Axotomy Day 2, decreased by 5% at Day 18 and 35 and increased by 3% at Day 60. These changes in Ψ_i which are small but statistically significant (paired t-test) may indicate that protein degradation as well as protein synthesis are affected in regenerating cranial nerve nuclei.
3. Effects of anesthetics on λ_i and $lCMR_{leu}$. λ_{WB} and Ψ_i determined in rats under thiopental are increased over control values indicating that there may be a decreased rate of protein degradation. Decreases (12 - 24%) in $lCMR_{leu}$ were found in eight of the 17 brain regions examined ($P < 0.05$). λ_{WB} determined in rats under ketamine anesthesia is unchanged from control. In a survey of $lCMR_{leu}$ in 36 brain regions, 20-26% decreases were found in 8 of the regions. It is noteworthy that both the prefrontal and frontal cortex were affected ($P < 0.01$) under ketamine anesthesia.
4. Effects of chronic cocaine treatment on $lCMR_{leu}$. Dr. Orzi has studied the effects of chronic and acute cocaine on $lCMR_{leu}$ in adult, male rats. Preliminary results suggest that cocaine treatment may result in small increases in $lCMR_{leu}$ in some brain regions.
5. Effects of "flooding" doses of L-valine on $lCMR_{val}$. In "flooded" and control rats $lCMR_{val}$ is being determined with the autoradiographic technique and appropriate regional values of λ_i calculated from regional values of Ψ_i determined under control and "flooded" conditions.

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, enzymatic and hormonal proteins. In addition, peptides that are considered to be neurotransmitters are in some, and possibly all cases, derived from the cleavage of large parent protein molecules.

Proposed Course:

1. Studies on the effects of ketamine anesthesia will be extended to examine regional heterogeneity in the value of λ_i.
2. Studies on the effects of chronic cocaine on $lCMR_{leu}$ will be completed. These studies will require evaluation of λ_i under the experimental conditions.
3. We propose to apply our quantitative approach to the study of brain protein synthesis to the study the effects of abnormalities in amino acid metabolism (such as PKU).

4. In the future we propose to examine the effects of brain leucine and α-ketoisocaproic acid levels on rates of protein synthesis and degradation.

Publications:

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2. Smith CBeebe. The measurement of regional rates of cerebral protein synthesis in vivo. Neurochem Res 1991;16:1037-1045.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 00903-15 LCM

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Purification of Brain Proteinases and Identification of Their Cleavage Products

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title,*

PI:	G.E. Deibler	Research Chemist	LCM, NIMH
Others:	T. Burlin	Biologist	LCM, NIMH

COOPERATING UNITS *(if any)*
Multiple Sclerosis Research Center, Georgetown University Medical Center, Washington, D.C. (J.R. Richert); Department of Neurology, Medical University of South Carolina (N.L. Banik)

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.0	0.2

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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.)*

Calcium activated neutral proteinase (CANP II) from bovine CNS myelin was isolated and purified by my collaborator, Dr. Banik. This enzyme was used to digest human myelin basic protein, component 1 (HBP-1) because the sequence of this protein is known. CANP digests were separated on HPLC into pooled fractions. N- and C-terminal sequences of these pools yielded eleven cleavage sties in HBP-1. The major site was Val(94)-Thr(95). Dr. Banik has also isolated and purified multicatalytic proteinase complex (MPC) from bovine brain. MPC also digests HBP-1 but at a much slower rate.

Human myelin basic protein (HBP) occurs in multiple forms. Three of these isoforms have been highly purified - HBP-1 (unmodified BP), 17.2kDa HBP (missing residues 106-116) and HBP 3pT98 (phosphorylated on Thr(98)). When compated with circular dichroism (CD) studies done on HBP-1, the CD studies of 17.2kDa HBP showed about 9% increase in ordered structure and those of HBP 3pT98 about a 12% increase. Both modifications of HBP-1, the delection of residues 106-116 and the addition of a single phosphate promoted secondary structure, probably an increase of β-structure.

Project Description:

Objectives:

1. Development of an HPLC program that will yield baseline separation between the peptides of CANP II and MPC digestions of HBP-1.

2. Determining the cytotoxic T-lymphocyte response to highly purified 17.2 kDa HBP and HBP 3pT98.

3. Development of sensitive N- and C- terminal methods that can be used for the analysis of purified peptides resulting from the digestion of HBP-1 with CANP II and HBP-1 with MPC at picomolar concentration.

Methods Employed:

1. Reversed-phase HPLC and ion-exchange HPLC will be used for the separation of peptides from the digests of HBP-1.

2. A sensitive amino acid analysis including all the amino acids plus tryptophan has been developed and is being used to quantitate the CANP cleavage products. For the protein synthesis project, the shortened amino acid analysis with collection of specific amino acids has been developed using fluorometric detection.

3. A limited thrombic digestion of HBP will be used to cleave all protein not phosphorylated at residue 98. The monophosphorylated HBP will be purified by fast protein liquid chromatography.

Major Findings:

The major CANP cleavage site, Val(94)-Thr(95) in human MBP was determined.
From the results of N-terminal sequencing and C-terminal sequencing of the HPLC pooled fractions, ten other cleavage sites were determined. (Sequencing done by Dr. Henry Krutzsch.)

MBP-specific human T-cell clones can be isolated from patients with multiple sclerosis and normal humans. By studying the proliferative response of the reactive T-cells from a multiple sclerosis patient, we have found forty MBP-specific human T-cell clones. Thirty clones responded to human myelin basic proteins (HBP) fragment 98-170, seven recognized fragment 1-97 and three did not proliferate in response to either fragment. In studies with xenogeneic MBPs isolated from rabbit, guinea pig, rat, cow and chicken, four different patterns of reactivity were seen with the clones which recognized 98-170, three with those that responded to 1-97 and three which

recognized neither fragment. The most common pattern of reactivity, expressed by eighteen of the clones, consisted of recognition of HBP fragment 98-170 and all xenogeneic MBPs tested with the exception of chicken MBP. There are possibly ten MBP-reactive sites on the human MBP. Two dominant sites of reactivity were found by their response to two synthetic peptides corresponding to residues 86-105 and 152-170. Synthetic peptide 152-170 also caused a cytotoxic T lymphocyte response in 19 clones which had produced vigorous proliferative responses.

Significance to Biomedical Research and the Program of the Institute:

Since CANP is associated with myelin and MPC is found in brain, they may have important implications in myelin metabolism and demyelinating diseases such as multiple sclerosis. Clarification of the role of CANP in myelin may uncover some important answers to the causes of demyelinating diseases.

While the direct correlation between BP-sensitivity and demyelination in multiple sclerosis (MS) patients has never been established, an autoimmune reaction to this antigen remains the most probable explanation for the development of MS. The current studies have shown that the T-cells of one MS patient demonstrate a very complex pattern of reactivity to MBP peptides. Similar studies in normals and other patients will clarify whether this T-cell reactivity is unique and related to disease activity.

Proposed Course:

Refinement of N- and C- terminal sequencing and development of better HPLC methods for the separation of the peptides of CANP and MPC digests of HBP-1, and the determination of the cleavage sites in HBP-1 for MPC.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02216-09 LCM

PERIOD COVERED

October 1, 1991 to September 30, 1992

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,*

PI:	L.J. Porrino	Guest Researcher	LCM,NIMH
Others:	L. Sokoloff	Chief	LCM, NIMH
	F. Pontieri	Visiting Fellow	LCM, NIMH
	A. (Tannenbaum) Crane	Research Biologist	LCM, NIMH

COOPERATING UNITS *(if any)*

Dept. of Pharmacology & Psychiatry, Boston Univ. School of Medicine, Boston, MA (C. Kornetsky); Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Winston-Salem, NC (S.I. Dworkin & J.E. Smith)

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Development Neurochemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	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.)*

This project is being terminated because most of the personnel on this project no longer work in the Labaratory.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02308-07 LCM

PERIOD COVERED
October 1, 1991 to September 30, 1992

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,*

PI:	B.F. Driscoll	Research Biologist	LCM, NIMH
Others:	V. Malinchik	Guest Researcher	LCM, NIMH

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	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.)*

Dissociated cell cultures of dopaminergic neurons from embryonic rat mesencephalon were grown in completely defined serum-free medium. We are using these cultures to describe and investigate the separate stages involved in excitotoxic cell death of neurons. Changing the medium of these cultures with medium containing 4.0 mM glutamine leads to complete destruction of the cultures. This damage is blocked by an antagonist (APV) of the NMDA-type of glutamate receptors. Excitatory amino acids (EAA) operating via NMDA receptors are responsible for culture damage. A low level of glutamate (μM) contaminating the large amount of glutamine is responsible for initiating damage; however, this NMDA-receptor event is finished in minutes after the medium change. Over the next 3-6 h the cells are damaged by unknown mechanisms; following this damage the level of glutamate in the extracellular medium rises to several hundred μM over the next 12 h. Most of this glutamate is generated extracellularly since it also appears if the medium is removed and recultured in wells with no cells. The most likely explanation is that damaged cells release glutaminase into the culture mediun which converts the extracellular glutamine to glutamate. If similar mechanisms operate in vivo, disintegrating neurons in a damaged area could generate large quantities of glutamate from the abundant glutamine in the cerebrospinal fluid; if healthy neurons were in the vicinity (as is found in vivo around a damaged area) this glutamate would subsequently damage these cells starting a cascade of ever widening cell damage and death.

Project Description:

Objective:

To determine the factors involved in development of central nervous system neurons by defining conditions responsible for survival and growth of these cells **in vitro.**

Methods:

Brains were removed from embryos and specific regions dissected out. Single cell suspensions were prepared from each region and cultured **in vitro.** The cells were cultured in serum-free medium which consisted of a mixture of Dulbecco's MEM medium (DMEM) with added insulin, transferrin and selenium (ITS).

Survival and degree of development of the mesencephalic dopaminergic neurons were determined by quantifying the level of the dopamine uptake system which is present in all dopaminergic neurons. The uptake of [^{3}H]-labelled dopamine was measured by liquid scintillation counting. Detection and quantifying of amino acids were performed using o-pthalaldehyde (OPA) derivitization and fluorescence analysis of the separated amino acids.

Major Findings:

We have continued our studies on the presence of NMDA receptors on cells in mesencephalic cultures. These cultures are severely damaged if the resident medium is exchanged for fresh medium containing 4.0 mM glutamine. This damage is blocked with low levels of a competitive inhibitor (2-amino-5-phosphonovaleric acid--APV) of NMDA-receptors indicating that the cell cultures are damaged by the action of excitatory amino acids (EAA) operating via these receptors. Recent studies demonstrate the presence of low levels of glutamate in the glutamine that is added to the tissue culture medium. The role of the NMDA-receptors is limited to a period of a few minutes following medium change when the glutamate contaminating the glutamine binds to NMDA-receptors. Damage to the neurons takes place over the next 3-6 h; the mechanisms responsible for damage are not known. After 3-6 h, the level of extracellular glutamate starts to rise and continues to rise until the cultures are assayed for the presence of viable dopaminergic neurons 20 h after medium change. The glutamate is generated in the extracellular medium and not inside the cells since removing the medium and reculturing it in the absence of cells does not affect the generation of the glutamate. If the medium is filtered (0.22 μm) or centrifuged (18,000 g) before reculturing without cells, no glutamate is generated. The medium contains a particulate fraction responsible for generating glutamate. The most likely candidate is the mitochondrial membrane-bound enzyme glutaminase. Following injury, damaged neurons apparently release intact mitochondria or mitochondrial fragments into the medium which then proceed to convert the ample supply of glutamine in the medium to glutamate.

Proposed Course:

Current studies are directed at determining the events that take place between ligand binding to the NMDA receptor and release of mitochondria or mitochondrial fragments into the medium. The former takes place within minutes of medium change and the latter between 3-6 h after medium change. In this interval, intracellular events initiated by NMDA-receptor binding lead to a level of neuronal damage so great and cell integrity is disrupted to such a degree that particulate material is released from the cells. In other systems, influx of extracellular Ca^{++} and release of intracellular stores of Ca^{++} have been implicated as playing a role in neuronal damage. Their role in the damage to these cultures will be examined. Also we will examine the effect of removing various noxious stimuli or adding various antagonists with time after the initiating events. This will allow determination of the length of time between when an event is initiated and when it's effects can be reversed. This is an important period of time since it is during this window of time that any practical clinical intervention would have to be made.

Significance to Biomedical Research and the Program of the Institute:

A significant amount of neuropathology arises following interruption of the supply of either nutrients or oxygen or both to the CNS. There is strong evidence implicating a role for the NMDA-receptor in the development of at least some of this pathology. Use of CNS cultures provides a valuable tool to determine the exact mechanisms responsible for neuronal damage and to select methods to block development of the damage.

Results derived from our studies on mesencephalic cultures should be applicable to any neurons expressing NMDA-receptors. However, these cultures are unique in that they contain dopaminergic neurons. These neurons, although few in number, project widely and influence a large number of CNS activities. In particular, premature loss or malfunction of dopaminergic neurons has been implicated as playing a role in some of the most devastating neurological disorders of humans. The cause of this neuronal loss remains a mystery.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02414-05 LCM

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Metabolic Interdependence of Neurons and Glia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title,*

PI: E. E. Kaufman Research Chemist LCM, NIMH

Others: B. F. Driscoll Research Biologist LCM, NIMH

COOPERATING UNITS *(if any)*

The R.W. Johnson Pharmaceutical Research Institute, Spring House, PA (Dr. Richard Shank)

LAB/BRANCH

Laboratory of Cerebral Metabolism

SECTION

Section on Developmental Neurochemistry

INSTITUTE AND LOCATION

NIMH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.85	0.6	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.)*

Work on the interdependence of neurons and glia has focused not only on CO_2 fixation in astroglial cells but also in ^{13}C-glucose metabolism in these cells. Astroglia, in culture, have been found to release products of CO_2 fixation into the surrounding medium with only a relatively small fraction of the products remaining in the cells. In mixed cultures of astroglial and neurons, however, approximately 90% of the labelled products were found within the cells suggesting that when these cells are in intimate contact there is a transfer of products from one cell to anther rather than a release of products into the extracellular medium. The anapleoric CO_2 fixation reaction occurring in glial cells may therefore serve to replenish essential metabolites in both neurons and astroglial cells.

PHS 6040 (Rev. 5/92)

Project Description

The overall objective of this project is demonstration of neuronal-glial metabolic interactions

Methods

Primary cultures of neuronal and astroglial cells have been prepared and grown according to standard procedures. Labeling with appropriate antibodies has been used to determine the purity of the cultures. Both fetal and newborn brains have been used as a source of these cells. CO_2 fixation has been measured by determining the acid stable [^{14}C] labeled products formed after incubation of cells with [^{14}C]$NaHCO_3$. Thin-layer and paper chromatography have been used to separate these products. The oxidation of [1-^{14}C] labeled pyruvate (to measure pyruvate dehydrogenase activity) will be measured by collection of the released $^{14}CO_2$ according to established procedures. The assay of C-AMP formation was assayed using the [125-I] scintillation proximity assay. In collaboration with Dr. Richard Shank, NMR is being used to identify the products of [^{13}C] glucose metabolism released by astroglial cell (in culture) into the medium. NMR will also be used to identify the products derived from CO_2 fixation which have been released by the astroglial cells into the medium.

Findings

It has been found that astroglial cells incubated in the presence of [^{14}C] labelled bicarbonate produce [^{14}C] labelled products which are then released into the surrounding medium. The amount of labelled product found within the cells is greater in the presence of high (25mm) K; the rate of release of these products into the surrounding medium is unaffected by the potassium concentration. Within the cells the labelled products appear to be mostly organic acids such as malate and sitrate with only a small fraction of the label in amino acids. In the extracellular fluid, however, the fractioning label in amino acids is equal to or exceeds the amount in organic acids. This suggests that the amino acids formed as a result of CO_2 fixation are being released into the medium whereas a larger fractin of the orgaic acids remain in the astroglial cell.

Significance to Biomedical Research and the Program of the Institute:

Pyruvate carboxylase in liver and kidney has been studied extensively and its role as the enzyme catalyzing the first step in gluconeogenesis is well established. The role of this enzyme

in brain is less well understood. For example, in brain its role may be mainly anapleoric rather than gluconeogenic. Patients with an inborn error of metabolism in which pyruvate carboxylase actively is either very low or missing have severe disorders of the central nervous system. There is a depletion of cerebral cortical neurons, gliosis and other degenerative changes such as marked reduction in cerebral white matter. Mental retardation, generalized seizures, and dystonic movements have also been observed. All of these observations suggest that this enzyme plays a critical role in the function of the central nervous system and that when this enzyme is missing severe problems develop.

Factors which control pyruvate carboxylase in brain have not been well characterized. It is known that potassium stimulates purified pyruvate carboxylase isolated from brain. We have examined the effect of varying the sodium and potassium concentrations in the medium in which astroglial cells are incubated. These experiments demonstrated that increasing the potassium concentration and lowering the sodium concentration will increase the rate of CO_2 fixation in astroglial cells.

There is evidence that the potassium released into the extracellular space following nerve stimulation is taken up by the neighboring astroglial cells. This uptake of potassium by astrocytes is believed to serve several purposes. One is simply to protect neurons against large changes in the concentration of extracellular potassium. Secondly, it has been proposed that astroglial cells may respond metabolically to changes in extracellular potassium and that the release of potassium by nerve cells may constitute a signal to the surrounding astroglial cells. Pentreath and Kai-Kai (Nature, 1982) have demonstrated an effect of both nerve stimulation and increased potassium on glycogen formation. Similarly, our results indicate that the anapleoric reaction catalyzed by pyruvate carboxylase may also respond to the increased potassium which results from nerve stimulation. Both of these reactions may be important in maintaining a constant energy supply and in replenishing the 3 and 4 carbon units necessary for the synthesis of the amino acid transmitters, GABA and glutamate in neurons as well as in astroglia.

Proposed Course:

The investigation of CO_2 fixation in astroglial cells will be continued. Primary emphasis will be placed on identification of the products of CO_2 fixation which are released from the astroglial cells. We will also attempt to demonstrate whether or not these products are transferred to neighboring neurons and, if so, how they are utilized by the neurons. A similar

investigation is being undertaken in collaboratin with Dr. Driscoll and Dr. Shank to determine the fate of [^{13}C] labelled glucose in both neurons and glial cells.

Publications

Kaufman, EE and Driscoll, BF. CO_2 Fixation in Neuronal and Astroglical Cells in Culture J. Neurochem 1992; 58: 258-262.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02431-05 LCM

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Intracellular Mechanisms of Carbohydrate Transport in Neurons and Glia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title,*

PI:	T. Nelson	Medical Officer (Research)	LCM, NIMH
Others:	L. Sokoloff	Chief	LCM, NIMH
	A. Tannenbaum	Biologist	LCM, NIMH

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	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.)*

All work on project # Z01 MH 02431-05 LCM has ceased. The microdialysis probe, which was central to the types of measurements used in this work, introduced severe and unacceptable artifacts which precluded accurate and meaningful measurement of steady-state glucose or deoxyglucose concentrations in rat brain. Part of the problem is explained by the development of edema in the region of the brain surronding the probe itself. Work by others has now shown that the sucrose space (which is used to estimate the extracellular space) is approximately 40% larger than it should be. Recent reports from several workers have confirmed my finding that the concentration of glucose in the extracellular space estimated from microdialysis fluid is much lower than the true concentration could possibly be.

Project Description

Objectives

The major objective of this project had been to gain a better understanding of how carbohydrates such as glucose and deoxyglucose are transported in glia and neurons and to compare how they are metabolized by these cells.

Brain dialysis experiments:

Methods Employed:

Brain microdialysis has been performed to determine the concentrations of deoxyglucose and glucose in brain extracellular fluid while the deoxyglucose concentration in the plasma is held at a constant level in normoglycemic rats.

By means of microdialysis it is theoretically possible to determine the steady-state concentrations of glucose, and other carbohydrates of interest, in brain, while the arterial concentration of the carbohydrate is maintained at some desired level. The concentration of the carbohydrate can be measured in the dialysate which leaves the probes. The concentrations found in the dialysate bears some relationship to the concentrations in the extracellular fluid (ECF) surrounding the probe. If the perfusion rate of fluid through the probe is sufficiently slow the concentration of small molecules, for which the dialysis membrane is permeable, would be imperceptibly less in the brain dialysate than the concentration that would have been found in the ECF in the absence of the probe. However, at these flow rates it would take too long to collect sufficient dialysate for analysis. Higher flow rates must be used, and at these flow rates the concentrations of small molecules in the dialysate may fall to 5 - 10% of their expected value in the ECF. Calibration of dialysis probes *in vitro* can be performed by determine the concentration in the dialysate which results from a given flow rate and concentration outside the probe. This does not offer a practicable solution for determining the concentrations in the ECF *in vivo*. The problem arises because the major barrier which prevents the molecules from diffusing is not the membrane itself but rather the tortuosity of the interstitial spaces in which the ECF exists, which impedes the progress of the molecule to the membrane. Lönnroth et al. addressed the problem of probe calibration *in vivo* in the periumbilical fat by perfusing the probe with several solutions of glucose with concentrations ranging from below to above the expected concentration in the ECF. By analyzing the dialysate and plotting the difference in concentrations in the perfusate these workers were able to calculate the concentration of perfusate which would give the same concentration in the dialysate. This concentration is assumed to represent the true concentration of glucose in the periumbilical ECF. Similarly it was assumed that in the microdialysis experiments performed in the current project - we would be able to calibrate our microdialysis probes *in vivo* by the method of Lönnroth. Numerous experiments have been performed in rats in which the plasma glucose has been held at an

approximately constant level while the dialysis probe was sequentially perfused with solutions of glucose ranging in concentration from less than to greater than that found in the whole brain. Such values of brain glucose have been determined by myself and others. Inasmuch as the intracellular concentration must be less than the concentration in the ECF since glucose is transported from the plasma into the ECF and thence into the cell where it is metabolized, the value found for the ECF glucose by the Lönnroth method can not be less than 20% of the plasma glucose concentration as predicted by the known distribution space for glucose in brain. In no instance was this true. All of the values determined by the Lönnroth method were less than those calculated from the plasma concentration and a distribution space of 0.2. The explanation for the discrepancy appears to be that at the flow rates chosen which varied between 1.0 and 2.5 μl/min the rate of removal of glucose from the ECF by the probe exceeded the rate of entry into the ECF from the plasma. The glucose concentrations in the ECF surrounding the probe were 10% to 75% of the expected values. Other workers who have employed microdialysis as a means to measure extracellular glucose have also begun reporting that the values they obtain are too low.

Until a method is found by which the concentration of substances in the ECF can be accurately determined from measurements on brain microdialysate it will not be feasible to determine intracellular concentrations of glucose and other carbohydrates using the indirect method originally proposed.

Significance to biomedical research and the program of the institute:

The present finding is of importance because it indicates that we cannot yet accept microdialysis as a method for measuring absolute concentrations of metabolites in the ECF. Microdialysis is a widely used technique, and there are many reports in which it has been used to determine the absolute concentration of compounds in the ECF. The technique of microdialysis is still useful when relative changes in concentration of substances in the ECF are to be measured.

Proposed course:

Work on the project which was originally described has been halted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02536-04 LCM

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Plasticity in the Central Nervous System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title,*

PI:	C.B. Smith	Research Chemist	LCM, NIMH
Others:	L. Sokoloff	Chief	LCM, NIMH
	P. Melzer	Visiting Fellow	LCM, NIMH
	Y. Sun	Visiting Associate	LCM, NIMH

COOPERATING UNITS *(if any)*
Dept. of Neurology, Johns Hopkins Medical School, Baltimore, MD (R. Tusa); Dept. of Otolaryngology, Johns Hopkins Medical School, Baltimore, MD (S. Herdman)

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	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 purpose of this project is to study the biochemical events associated with development, plasticity and involution of the nervous system. We have used the quantitative autoradiographic [1-^{14}C]leucine method to study the sites of origin and the process underlying changes in nervous system organization that take place during these events and the [^{14}C]deoxyglucose method to examine the outcome of the changes, i.e. the functional reorganization that has occurred. Our studies of normal development in rats show decreasing rates of protein synthesis in most brain regions from day 14 to the adult stage except in the paraventricular and supraoptic nuclei of the hypothalamus. We are studying two model systems of plasticity: (a) the developing monkey visual system, and (b) the developing and adult mouse somatosensory system. Results of our studies of chronic monocular deprivation in monkeys early and late in the critical period show that the biochemical response that may underlie the functional reorganization of the striate cortex is a decrease in the rate of protein synthesis indicative of a decrease in the growth rate in the deprived geniculate cells. Results of deoxyglucose studies on the effects of whisker follicle removal in neonatal mice show that when the lesioned mice reach adulthood metabolic maps in both somatosensory cortex and in trigeminal brainstem nuclei are altered. When adult mice are similarly lesioned metabolic maps in the cortical barrel field are also found to be altered after 160 days. These results indicate functional reorganization in both the neonatal and the adult whisker-barrel pathway in the mouse and they suggest that this system may serve as a useful model of both developmental and adult plasticity.

Project Description:

Objectives:

1. To study the metabolic consequences of aging.
2. To study the biochemical events associated with plasticity.

The specific aims pursued during this fiscal year are:
1. Effects of developmental age and normal aging in rats on λ_{WB} and on $lCMR_{leu}$.
2. To study the effects of whisker follicle removal on $lCMR_{glc}$ in the whisker-to-barrel pathway of the mouse.

Methods Employed:

1. Determination of local rates of cerebral protein synthesis ($lCMR_{leu}$).
   Animals are administered i.v. L-[1-^{14}C]leucine (100 μCi/kg), timed arterial blood samples are drawn for determination of the time course of plasma leucine specific activity and at 60 min the animal is killed. Free [^{14}C]leucine in tissue sections is removed by fixation and washing in 4% formalin. Concentrations of [^{14}C]protein in brain regions are determined by quantitative autoradiography. These values, the calculated integrated specific activity of [^{14}C]leucine in the tissue, and λ_i are used in the operational equation of the method to calculate $lCMR_{leu}$.
2. Determination of λ_i.
   λ_i is the ratio of the steady state distribution ratios for labeled and unlabeled leucine between the tRNA-bound pool in the tissue and arterial plasma. A pure tRNA-bound amino acid fraction is extracted from brain after rats are maintained for 60-90 min in a steady state for labeled leucine with a programmed infusion. The specific activities of this fraction from brain and the acid-soluble fraction from arterial plasma are determined and value of λ_i is calculated.
3. Determination of local rates of cerebral glucose utilization ($lCMR_{glc}$).
   Animals are administered i.v. [^{14}C]deoxyglucose (120 μCi/kg body wt) and timed arterial blood samples are drawn for determination of the time courses of plasma levels of glucose and [^{14}C]deoxyglucose. At 45 min the animal is killed and tissue sections of brain are prepared for autoradiography. Concentrations of [^{14}C] in brain regions determined by quantitative autoradiography, calculated final tissue concentrations of [^{14}C]deoxyglucose, calculated ratio of tissue concentrations of [^{14}C]deoxyglucose and glucose integrated over the experimental interval, and the lumped constant are used to calculate $lCMR_{glc}$.
4. Removal and stimulation of mouse whisker follicles.
   The follicles of whiskers C_1, C_2, and C_3 on the left muzzle were surgically removed from both adult and neonatal mice. Mice with neonatal lesions were allowed to reach adulthood before study. The mice lesioned during adulthood were studied at 2, 4, 8, 64, 160, and 300 days after the lesion. For the $lCMR_{glc}$ studies pieces of fine metal wire

were glued to whiskers B_{1-3} and D_{1-3} on the left muzzle; all other whiskers were clipped. Whiskers were stimulated during the study by means of a pulsing magnetic field.

Major Findings:

1. Effects of developmental age. The mean ± SEM value of λ_{WB} in rats at 7, 10, 14, 21, and 35 days after birth was determined to be 0.43 ± 0.03, 0.45 ± 0.02, 0.52 ± 0.01, 0.53 ± 0.02 and 0.58 ± 0.01, respectively. Values are lowest in the 7 day old rats and increase with age until 35 days at which time the value of λ_{WB} is the same as that of adult rats. These results show that early in development a greater fraction of the precursor leucine pool is derived from protein degradation. These values of λ_{WB} are now being used in the determination of $lCMR_{leu}$ in developing rats with the autoradiographic [1-^{14}C]leucine method. These results will be reported at the 1992 meeting of the Society for Neuroscience in Anaheim.
2. Effects of normal aging in rats on λ_{WB}. We have reported previously (Ingvar et al., *Brain* (1985), **108**, 155-170) that there are decreases in $lCMR_{leu}$ which occur in the brain as a whole and in selective brain regions; we assumed that the value of λ_{WB} was 1.0 and that it did not change with age. Now we are evaluating the effect of age on λ_{WB}. Preliminary results in two middle-aged and one aged rat suggest that it does not appear to change with aging.
3. Effects of whisker follicle lesions in neonatal mice on $lCMR_{glc}$. We have examined the functional and morphological consequences in adult mice of removal of follicles C_{1-3} during the first few days of life. Unilateral stimulation of whiskers B_{1-3} and D_{1-3} in control and lesioned mice results in increased $lCMR_{glc}$ in the two discrete representations of these whiskers in the contralateral cortical barrel field and in two trigeminal brainstem nuclei on the ipsilateral side.. In the lesioned mice, $lCMR_{glc}$ was also elevated in the vacant territory between the rows (what would have been barrels C_{1-3}) in both cortex and in subnucleus interpolaris. We hypothesize that the ganglion cells serving the still intact follicles surrounding the lesion must have innervated the vacant territory in the brainstem. This reorganization may induce the observed changes in barrel cortex. Manuscripts describing these changes are in preparation.
4. Effects of whisker follicle lesions in adult mice on $lCMR_{glc}$. Preliminary results show that stimulation of whiskers B_{1-3} & D_{1-3} on the lesioned side in mice $\geq$160 days after the lesion result in changes in $lCMR_{glc}$ similar to the changes that we observed in mice with a neonatal lesion. In the adult studies, however, no morphological changes have been found in the whisker representations.

Significance to Biomedical Research and 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 neurochemists the world over. In an attempt to study some of the biochemical processes underlying plastic changes, we have embarked on these studies of the mouse somatosensory system about which the physiology and anatomy are well

known. Studies with the [^{14}C]leucine method for $lCMR_{leu}$ and the [^{14}C]deoxyglucose method for $lCMR_{glc}$ are directed at a description of some of the biochemical events which occur and a determination of the regulation of these events. The understanding of these events may provide insight into the unique properties of the critical period which make it so responsive to environmental manipulation and the parameters that determine which parts of the nervous system maintain this capability of reorganization into adult life. Insofar as aging is rapidly becoming a problem of increasing social significance, research focused on senescent changes in the ability of the brain to function, may be of considerable importance to the medical community. Our results indicate that some of the changes which occur in the nervous system with age may be the consequences of a decreased functional activity. Further understanding of the basic biochemical processes underlying plastic changes in the nervous system of either an involutional or developmental nature, may be useful in trying to prevent and/or reverse such senescent changes.

Proposed Course:

1. Results of studies of protein synthesis in normal development in rats are currently being analyzed. A manuscript reporting these results will be prepared.
2. Studies of the effects of normal aging will be extended to include more animalsp.
3. Results of studies of plasticity in the mouse whisker-to-barrel pathway are being analyzed and several manuscripts are in prepartation. Eventually we hope to extend these studies to look for an effect between 64 and 160 days post-lesion in the relay stations of the whisker-to-barrel pathway in brainstem and thalamus on $lCMR_{leu}$.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02569-02 LCM

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Kinetic Modeling of Tissue Heterogeneity in Metabolism and Blood Flow Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title,*
PI: K. Schmidt Computer Systems Analyst LCM, NIMH

COOPERATING UNITS *(if any)*
PET Center, Hospital San Raffaele, Milan, Italy (G. Lucignani, M.D., F. Turkheimer)

LAB/BRANCH
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SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	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.)*

Due to the limited spatial resolution and partial volume effects of positron emission tomography (PET), most tissue regions studied with PET are heterogeneous with respect to the physiological and/or biochemical processes being examined and with respect to the concentrations of the relevant labeled compounds in the tissue. All quantitative work up to now has made the simplifying assumption that the tissues are homogeneous, and serious misinterpretations of results obtained by use of these methods have occurred. We have studied the effects of tissue heterogeneity on determination of local cerebral glucose utilization. Mathematical models to describe the kinetics of deoxyglucose or flurordeoxyglucose uptake and metabolism in heterogneous tissues were developed and validated in simulation, animal, and human studies. The most appropriate kinetic model and optimal experimental protocol for the measurement of cerebral glucose utilization in man with [^{18}F]fluorodeoxyglucose and PET were identified.

We are currently developing new techniques for analysis of time-series data from tracer studies in which the underlying kinetic models have not yet been established. These techniques will enable us to build appropriate kinetic models of new tracers and to extend mathematical models of currently used tracers to additional physiological and pathophysiological conditions.

Project Description:

Adaptations of the [^{14}C]deoxyglucose method for the quantitative determination of local rates of cerebral glucose consumption and of the [^{14}C]iodoantipyrine method for determination of local rates of cerebral blood flow for human use with PET and [^{18}F]fluorodeoxyglucose and ^{15}O-labeled water, respectively, have presented some special problems. Due to the limited spatial resolution and partial volume effects of PET scanners, measurement of radioactivity in homogeneous tissues is rarely, if ever, achieved. Hence one of the explicit assumptions of both methods, that the tissues to which they are applied are homogeneous with respect to the physiological and/or biochemical processes being examined and with respect to the concentrations of the relevant labeled compounds in the tissue, is not satisfied. As a result, serious misinterpretations of results obtained by use of these methods have occurred. We have systematically examined the effects of tissue heterogeneity on the measurement of cerebral blood flow and glucose utilization, developed and validated kinetic models to describe the uptake and metabolism of deoxyglucose or fluorodeoxyglucose in heterogeneous tissues, and determined the optimal time frame and analytical procedure to be used for the measurement of cerebral glucose utilization in man with [^{18}F]fluorodeoxyglucose and PET. We are currently developing additional analytical methods to evaluate the kinetic behavior of other tracers used with PET.

Effects of Heterogeneity on Determination of Local Cerebral Glucose Utilization.

K. Schmidt, in collaboration with G. Lucignani (PET Center at San Raffaele Hospital, Milan, Italy), has studied data from normal human subjects administered a pulse of [^{18}F]fluorodeoxyglucose to measure cerebral glucose metabolic rates with PET. The effects of tissue heterogeneity on the estimates of kinetic model rate constants and on calculated regional cerebral glucose utilization were evaluated. Results obtained from the PET studies were consistent with the results previously obtained in simulation studies performed in this laboratory, i.e. when kinetic models designed for homogeneous tissues were applied to heterogeneous tissues, estimates of the rate constant for efflux of [^{18}F]fluorodeoxyglucose from the tissue, k_2^*, and the rate constant for phosphorylation of [^{18}F]fluorodeoxyglucose, k_3^*, initially fell sharply with time and became relatively constant later in the experimental period. Estimates of the rate constant for dephosphorylation of [^{18}F]fluorodeoxyglucose-6-phosphate, k_4^*, in large regions of interest were significantly greater than zero and fell continuously as the duration of scanning increased up to 120 min following the pulse of [^{18}F]fluorodeoxyglucose. Goodness-of-fit was found to be an insufficient criterion for the selection of a kinetic model, and large estimates of k_4^* were shown to be insufficient evidence to conclude that there is dephosphorylation of [^{18}F]fluorodeoxyglucose-6-phosphate in the cerebral tissue. In fact, use of the homogeneous tissue kinetic model that includes desphosphorylation led to overestimation of whole brain glucose utilization by more than 20% when compared with glucose consumption rates usually obtained by the model independent Kety-Schmidt Technique. Glucose utilization was accurately estimated by the tissue heterogeneity model and, in

experimental periods sufficiently long to minimize the effects of tissue heterogeneity, also by the original kinetic model of the deoxyglucose method. Results from these studies were presented at the 1991 European Association of Nuclear Medicine Congress and been published in the Journal of Cerebral Blood Flow and Metabolism (Schmidt et al., 1992).

Optimal Protocol for Measurement of regional Cerebral Glucose Utilization with [18F]fluorodeoxyglucose and PET in Heterogeneous Tissues.

Results from the previous study demonstrated that one of the tissue kinetic models that is now widely used with [^{18}F]fluorodeoxyglucose and PET overestimated cerebral glucose utilization since it assumes both a homogeneous tissue and dephosphorylation of [^{18}F]fluorodeoxyglucose-6-phosphate. The kinetic models that include no such dephosphorylation term were shown to accurately estimate the rate of cerebral glucose utilization provided that they were used in an appropriate time interval (Schmidt et al., 1992). K. Schmidt, in collaboration with G. Lucignani (PET Center at San Raffaele Hospital, Milan, Italy), undertook a study to establish an optimal, yet practical, time frame and procedure that minimizes errors due to tissue heterogeneity in the determination of regional cerebral glucose utilization. The original kinetic model of the deoxyglucose method, because it provides accurate estimates of glucose utilization at long times following the pulse of [^{18}F]fluorodeoxyglucose and is computationally much simpler than the tissue heterogeneity model, was evaluated with both dynamic (multiple-scan) and autoradiographic (single-scan) procedures. In addition, the multiple-time graphical analysis technique of Patlak was examined. The Patlak technique applies to heterogeneous tissues as well as to homogeneous tissues but is limited by the necessity to identify both a lower time limit at which all tissue pools have equilibrated with the arterial plasma and an upper time limit before significant product loss occurs. Results of the study showed that the Patlak procedure provided estimates of glucose utilization that were relatively constant with time when tissue data acquisition started not earlier than 45 after tracer injection; data acquisition could be continued up to 120 min after tracer injection without significant effects of product loss. Results of the study also showed that best estimates of the mass-weighted average kinetic model rate constants were those determined over an experimental period beginning at the time of tracer injection and continuing for a minimum of 60 min and a maximum of 120 min; rate constants determined over shorter experimental periods overestimated their true mass-weighted averages. Hence dynamic procedures, because they require the determination of kinetic model rate constants, should not be carried out over experimental periods shorter than 60 min. The autoradiographic procedure, on the other hand, provided stable estimates of glucose utilization with a single 10-15 min scan carried out at any time between 30 and 120 min after tracer injection, provided that an appropriate set of population average rate constants was used. The period between 60 and 120 min following tracer injection combined with the autoradiographic procedure was found to be optimal for quantitative glucose utilization studies. A major advantage suggested by these findings is the possibility of using doses of [^{18}F]fluorodeoxyglucose lower than those currently employed

and compensating for lower counting rates in the tissues by increasing the duration of the tissue scanning interval. Results from this study were presented at the European Association of Nuclear Medicine Congress 1992.

Development of the Spectral Analysis Technique for Evaluating Kinetic PET Data.

All compartmental analyses that have been used to date to describe the kinetics of tracer uptake and metabolism in the cerebral tissues have been based on the a priori definition of a kinetic model and nonlinear least squares algorithms to estimate the parameters of the model from the time courses of radioactivity measured in any given region of interest in a PET study. Errors in the a priori kinetic model may lead to erroneous conclusions from the model predictions. For example, we have shown that calculated rates of glucose utilization are overestimated in [^{18}F]fluorodeoxyglucose studies when a homogeneous tissue kinetic model that includes dephosphorylation of [^{18}F]fluorodeoxyglucose-6-phosphate is applied to heterogeneous tissues (Schmidt et al., 1992). Recently, a spectral analysis technique to identify the components in PET tissue radioactivity data with no prior specific model assumptions has been proposed. K. Schmidt, in collaboration with F. Turkheimer and G. Lucignani (PET Center at San Raffaele Hospital, Milan, Italy), has implemented the spectral analysis technique and optimized the procedure for the measurement of glucose utilization in brain with [^{18}F]fluorodeoxyglucose and PET. Results obtained thus far indicate that the optimized procedure provides values for glucose utilization that are constant with time and very close to rates determined by other techniques when the latter are applied in an appropriate time interval. We are currently examining the possibility of applying the spectral analysis technique to the evalution of kinetic PET data for various receptor ligands. Preliminary results from these studies have been presented at the Society of Nuclear Medicine 39th Annual Meeting.

Proposed Course:

The effects of tissue heterogeneity on the results obtained with currently available methods for measuring regional cerebral blood flow and metabolism in man with PET will be studied further. New techniques for analysis of time-series data will be further developed and applied to tracer studies in which the underlying kinetic models have not been previously established.

Publications:

Schmidt K, Lucignani G, Moresco RM, Rizzo G, Gilardi MC, Messa C, Colombo F, Fazio F, Sokoloff L. Errors introduced by tissue heterogeneity in estimation of local cerebral glucose utilization with current kinetic models of the [18F]fluorodeoxyglucose method. *J Cereb Blood Flow Metab* 1992 12:823-834

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02570-02 LCM

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Studies on the Metabolism of Neurons and Astroglia in Tissue Culture

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title,*

PI: B.F. Driscoll Research Biologist 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 STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	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.)*

Perturbation of the metabolism of the brain has serious negative effects on the well-being and survival of the organism. The contribution of the various cell-types in the brain to overall metabolism cannot be determined by in vivo experiments. Growth of these sub-populations separately in tissue culture permits examination of their metabolism independently. Furthermore, the effect of varying the extracellular concentration of glucose to mimic conditions seen in vivo in pathologic conditions can be assessed on the separate cell populations. Results from such studies demonstrate that neurons and astroglia handle 2-[^{14}C]DG in a similar fashion under normoglycemic (1.0 - 2.0 mM glucose), slightly hyperglycemic (5.0 mM glucose) and hypoglycemic (0.1 - 0.5 mM glucose) conditions; the intracellular:extracellular distribution of 2-[^{14}C]DG is similar in both cell types. Results from studies on glucose were more complex due to the presence (especially in astroglia) of an intracellular pool of glucose apparently separate from the metabolically active pool. Thus, during periods of hypoglycemia, the ability of neurons and astroglia to transport glucose into the cell's metabolically active pool does not appear to differ.

Project Description:

Objective:

To use cultures of various central nervous system cell types to study the metabolic properties of these cells **in vitro** with particular emphasis on developmental changes and induced changes that could be related to pathologic sequelae **in vivo.**

Methods:

Brains are removed from embryonic day 16 rat embryos and the striatum dissected out. Single cell suspensions are prepared and the cells cultured **in vitro** in medium containing 10% fetal bovine serum. Cultures consisting of predominantly neurons, astroglia or fibroblasts are prepared.

The metabolic state of cells is assessed in several ways. To determine the distribution space of glucose and 2-[^{14}C]DG, the various cell types are labeled with 2-[^{14}C]DG (and [^{3}H]sucrose as an extracellular marker), frozen and extracted. The extracts are fractionated on anion-exchange HPLC columns to identify and quantify the sugars and their metabolic products. Separate cultures are used to determine the intracellular volume of the cells in culture. To simply quantify metabolism, cells are labeled with 2-[^{14}C]DG, followed by an appropriate period to allow for efflux of non-metabolized 2-[^{14}C]DG, and the level of 2-[^{14}C]DG-6-P in the cells determined.

Major Findings:

Initial studies were done to determine the time of influx and efflux of glucose and 2-[^{14}C]DG in both neurons and astroglia in order to determine the time period necessary to establish steady-state conditions under various conditions and the time needed to remove unreacted metabolites from the cells. This large body of data was used to design studies on the distribution space of glucose and 2-[^{14}C]DG in neurons and (separately) astroglia under conditions ranging from extreme hypoglycemia (0.1 mM glucose) to hyperglycemia (20 mM glucose). The astroglia and neurons appear to transport 2-[^{14}C]DG in a similar fashion under all conditions. The situation with glucose is more complicated since their appears to be a pool of intracellular glucose in astroglial cultures, and possibly also in neuronal cultures, which is not in equilibrium with the metabolically active glucose pool. While not particularly evident at the normoglycemic level of glucose, at hypoglycemic levels it is in sufficient concentrations to raise the intracellular levels of glucose well above that found outside the cell.

Proposed Course:

Currently we are attempting to determine if the pool of intracellular glucose that is separate from the metabolically active glucose pool is real or an artefact. If real, we will determine the function of the pool and conditions that might determine it's magnitude.
Studies are also underway to examine the nature of the metabolic interactions between neurons and astroglia. Collaborative studies are underway with Elaine Kaufman in our lab on CO_2 fixation by astroglia and the mechanisms which stimulate this reaction. We are also attempting to identify the products of CO_2 fixation and determine their metabolic fate in astroglial/neuronal co-cultures. Dr. Kaufman and I are also collaborating with Richard Shank at the R.W. Johnson Pharmaceutical Research Institute on identifying (using NMR) the products of [^{13}C]glucose metabolism by various types of CNS cultures.

Significance to Biomedical Research and the Program of the Institute:

Information on the metabolism of neurons and astroglia, particularly under moderate to severe hypoglycemic conditions, is useful when attempting to determine mechanisms by which cells cope or fail to cope with these non-normal physiologic (i.e. pathologic) situations. Knowledge of the nature of these control mechanisms will be useful in developing strategies for intervening in the events that take place in the brain when metabolism is compromised. In particular, it is important to determine whether there is beneficial metabolic cooperation between astroglia and neurons under these conditions and, if there is, whether it can be enhanced. Clearly, in humans the result of altered metabolism due to abnormal circumstances can have devastating effects on the function of the CNS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02590-02 LCM

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Nitric Oxide in Local Cerebral Blood Flow

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title,*

PI:	C. Kennedy	Medical Officer(Research)	LCM, NIMH
	T. Nelson	Medical Officer(Research)	LCM, NIMH
Others:	K. Adachi	Visiting Fellow	LCM, NIMH
	A. (Tannenbaum) Crane	Biologist	LCM, NIMH
	F. Wang	Guest Researcher	LCM, NIMH
	S. Takahashi	Visiting Fellow	LCM, NIMH

COOPERATING UNITS *(if any)*
Laboratory of Neurochemistry (S. Kaufman and K. Campos)

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Developmental Neurochemistry

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.75	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.)*

This project, begun last year, is given to an evaluation of the role of nitric oxide as a potential mediator of the coupling of local cerebral blood flow to local cerebral metabolic and/or functional activities. Having found that the action of 5% CO_2 to increase cerebral blood flow was unaffected by the intravenous administration of methylarginine in doses up to 100 mg/kg, the possibility was considered that CO_2 acted on the abluminal side of cerebral vessels and that the inhibitor must therefore cross the blood-brain barrier to block nitric oxide synthase. After demonstrating by direct chemical analysis that methylarginine crosses the barrier relatively slowly, nitroarginine methyl ester, a better candidate for gaining access to brain from plasma was evaluated. This more powerful inhibitor of nitric oxide synthase, also failed to block an increase in cerebral blood flow by 5% CO_2. Both methylarginine and nitroarginine were then examined for an effect on the increase in local blood flow induced by functional activity. In experiments during which whiskers were repeatedly stroked on one side, neither inhibitor given intravenously reduced the blood flow response in any part of the pathway from brainstem to barrel field of the cortex. Intracisternally administered nitroarginine likewise was without effect in blocking the blood flow response to stroked whiskers. On the basis of the results obtained to date it appears that nitric oxide is unlikely to be a significant mediator of either the action of CO_2 or of functional activity to increase cerebral blood flow.

Project Description:

Objectives and Rationale:

The rationale behind the undertaking of this project was presented in detail in the Annual Report for 1991. Briefly, it has been known for many years that cerebral vasculature is capable of adjusting blood flow to meet the continually changing local energy requirements of the brain. In increase in energy metabolism results increased pCO_2, decreased pO_2, increased H^+, increased K^+ adenosine release, each of which have been shown to cause vasodilation. Each is tonically active so that when altered in the opposite direction they cause vasoconstriction. Thus it is widely assumed that these products, alone or in concert, account for the coupling of local blood flow to neuronal activity. However the mechanism underlying this linkage is not understood. The disclosure of a continuously liberated vasodilator from the endothelium of virtually all systemic blood vessels by Furchgott over a decade ago instigated a world wide research effort to learn the origin and identity of this Endothelium-derived Relaxing Factor (EDRF). This factor is now known to be nitric oxide, a short-lived (half life 5-10 seconds) gas which is continuously liberated from endothelial cells in a reaction between arginine and a Ca^{++}, and calmodulin-dependent monooxygenase. It diffuses to adjacent smooth muscle to activate guanylic cyclase. The rise in cyclic GMP leads to relaxation of muscle in the vessel wall and vascular dilatation. The possibility was considered that nitric oxide may be the missing link in our present knowledge of the manner in which blood flow in brain is coupled to local functional activity. The studies are being done in collaboration with S. Kaufman and K. Campos of the Laboratory of Neurochemistry, NIMH, who will be responsible for the enzymological aspects of these studies.

Methods Employed:

The administration of a competitive blocker of nitric oxide synthase was chosen as a way of evaluating nitric oxide's role in experimental conditions which result in an increase in cerebral blood flow . A reduction in the blood flow response to induced functional activity in the presence of the inhibitor would be evidence of its role in coupling the blood flow response. Inhibitors evaluated were: N^G-monomethyl-L-arginine and N^ω-nitro-L-arginine methyl ester. Local cerebral blood flow was measured with the [^{14}C]iodoantipyrine method, local cerebral glucose utilization with the [^{14}C]deoxyglucose method.

Unilateral stroking of the whiskers was chosen as the mode of stimulation for the studies on the response of local blood flow to induced functional activity. The fact that the pathway from the trigeminal nuclei on one side to barrel field of the cortex remains unilateral even after crossing in the midbrain made it possible to assess the effect of inhibitors of nitric oxide synthase on blood flow by comparing right to left differences in any part of the whisker-to-barrel pathway. Four places were selected: the spinal nucleus of V, the principal nucleus of V, VPM of the thalamus, and the barrel field of the somatosensory cortex. The inhibitors were evaluated for their effects both after being given intravenously and intracisternally.

T. Nelson and A. Tannenbaum have continued examining these same questions with a different approach. They have employed a microdialysis probe placed in the cerebral cortex to administer penicillin and thereby evoke focal seizure activity. The local blood flow accompanying the seizure activity was assessed by means of the [^{14}C]iodoantipyrine method. Experiments in which nitric oxide synthase inhibitor, methylarginine, was incorporated with penicillin in the dialysis infusion on one side and penicillin alone in the infusion on the other side provided a means of determining any role played by nitric oxide in the vascular response.

Major Findings:

In the previous year it was found that hypercapnea-induced increases cerebral blood flow were not diminished by the intravenous administration of methylarginine in a wide range of doses. The possibility existed that this was due to the fact that the methylarginine failed to gain access to the site where nitric oxide might also be liberated, possibly the abluminal wall. Therefore, experiments were undertaken to determine whether the methylarginine crossed the blood brain barrier. The results indicated that such penetration was very slow. Only about 7% of the inhibitor entered the brain in a ten minute period following its intravenous administration in a dose of 100 mg/kg. Therefore, nitroarginine methyl ester, having a structure indicating that it might cross the blood-brain barrier more readily, was therefore evaluated. As had been found with methylarginine, nitroarginine methyl ester also failed to reduce the effect of 5% CO_2 to increase cerebral blood flow.

Monomethyl arginine was without effect on local glucose utilization in any region of brain.

In the evaluation of the role of nitric oxide in the modulation of local blood flow accompanying functional activity (stroking of whiskers) no effect was observed of either inhibitor whether they were given intravenously or directly into the subarachnoid space i.e. the cisterna magna.

Proposed Course

The failure to detect any effect of methylarginine on the vascular responses to CO2 and to functional activity may be in part to its limited penetration of the blood brain barrier. To supplement the studies already conducted in which chemical procedures were used, ^{14}C labelled methylarginine is presently being synthesized by special order (Cal Bionuclear) for use in more definitive experiments to learn of the distribution of methylarginine between blood and brain.

Significance to Biomedical Research and the Program of the Institute

The mechanisms of regulation of cerebral blood flow by cerebral energy metabolism and functional activity have represented for almost a century a major area of research of fundamental importance to cerebral physiology. It is not only a fruitful area to investigate

the pathogenesis of a number of nervous diseases, but an understanding of these mechanisms is essential to interpret properly the functional significance of changes in lCBF being observed with current PET imaging techniques in studies of psychiatric diseases and cognitive functions.

Publications

The work was presented at the 4th International Symposium on Pharmacology of Cerebral Ischemia in Marburg, Germany this July. Manuscripts are presently being drafted for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02624-01 LCM

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Role of GHB in Metabolic Regulation at the Cellular Level

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title,*

PI:	T. Nelson	Medical Officer (Research)	LCM, NIMH
Others:	E.E. Kaufman	Research Chemist	LCM, NIMH
	B.F. Driscoll	Research Biologist	LCM, NIMH

COOPERATING UNITS *(if any)*
None

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Laboratory of Cerebral Metabolism

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	

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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.)*

This report describes a new project in which the biological role of γ-hydroxybutyrate (GHB) is being investigated. GHB is an endogenous compound which was first found to be present in brain in concentrations ranging from 1 - 4 μM, but which was later found to be widely distributed throughout the other organs of the body. Pharmacological doses of this hydroxyacid produce catatonic behavior in rats and dose-dependent reductions in the rate of glucose utilization in brain. These effects, and others reported in most of the previous studies, have been elicited by doses of GHB that would produce tissue concentrations of the compound many hundreds of times higher than that which normally exists in brain. Initial studies in this laboratory have shown that concentrations of GHB as low as 1 μM can alter calcium metabolism in astrocytes cultured from 16 day rat fetuses. The mechanism by which calcium metabolism is altered in these cells is the first objective in this project

Project Description

Objectives

The major objective of this project is to gain a better understanding of the role GHB plays at physiological concentrations in astrocytes and neurons. Does this compound play a significant role in the regulation of any normal metabolic processes in excitable cells such as neurons or in supportive cellular elements such as astrocytes? More than 30 years of investigation have established that high doses of this compound block dopaminergic neurons and inhibit processes that are controlled by these neurons. Several reports show that the opiate antagonist, naloxone, blocks many of the responses seen with high doses of GHB. There are no reports on the mechanisms by which the biochemical effects produced by very low concentrations of GHB occur, though low doses of this compound have been found to affect temperature regulation and to cause the secretion of growth hormone and prolactin. The effect of GHB on processes such as calcium metabolism which are known to be involved in the regulation of excitable and non-excitable cells will be studied to see if the process is affected by GHB. Where GHB is found to have an effect the underlying mechanism of the effect will be investigated. Preliminary studies have shown that GHB does alter ^{45}Ca uptake in astrocytes at concentrations as low as 1 μM. One of the first objectives of this project will be to determine the mechanism by which GHB exerts this effect.

Methods Employed:

Initial studies will be performed with ^{45}Ca which can measure the uptake or release of calcium from the cell through all calcium channels. Further studies will be made with fluorescence measurements with calcium-binding probes such as Fura-2 to measure the effect of GHB on the concentration of free calcium within the cell. Channel blocking agents will be used to determine whether GHB has any effect on the calcium channels, and pertussis and cholera toxins will be used to see whether GHB affects the G proteins.

Significance to biomedical research and the program of the institute:

For many years GHB has been claimed to be a neuromodulator or even a neurotransmitter; the present project aims to determine whether, indeed, GHB plays a significant biological role at physiological concentrations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00507-10 LCM

PERIOD COVERED

October 1, 1991 to September 30, 1992

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,*

PI:	R. M Cohen	Guest Researcher	LCM, NIMH
Others:	A. J. Zametkin	Senior Staff Psychiatrist	LCM, NIMH
	D. J. Doudet	Visiting Associate	LCM, NIMH
	J. A. Matochik	Research Psychologist	LCM, NIMH

COOPERATING UNITS *(if any)*

Clinical Center, Nuclear Medicine, NIH (R.E. Carson); Geriatric Psychopharmacology, NIMH (T. Sunderland); Child Psychiatry Branch, NIMH (T.C. Gordon, J.M. Rumsey, J.L. Rapoport); Neuropsychiatry, NIMH (A. Elkashef)

LAB/BRANCH

Laboratory of Cerebral Metabolism

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Section on Clinical Brain Imaging

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
8.5	4.5	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 Section on Clinical Brain Imaging has made a sustained effort to use positron emission tomography (PET) to help elucidate the neural mechanisms and pathophysiology of psychiatric disorders. The major focus of the Section has been directed at using PET to address questions about Attention-Deficit Hyperactivity Disorder (ADHD) in adult and teenage subjects. PET studies related to ADHD were conducted in three areas:

Stimulant effects Studies were completed on the cerebral metabolic effects of acute and chronic dextroamphetamine and methylphenidate treatment. Both stimulants appear to have modest metabolic effects in the brain while producing positive behavioral improvement.

Development PET studies on normal teenagers and those with ADHD and schizophrenia are continuing. A new method, which allows minors to be scanned with miminal radiation, is being employed and the initial description of the procedure has been accepted for publication. In addition, teenagers with schizophrenia are being scanned by our Section using the new method.

Thyroid Resistance Symptoms of ADHD are highly associated with generalized resistance to thyroid hormone, a disorder with a defined genetic defect. PET studies show impaired attention and reduced metabolism in the parietal lobe.

The Section is continuing studies on scopolamine as a model for the memory impairment in Alzheimer's Disease. Initial results show altered metabolism in brain areas related to memory function. The Section is continuing work on the development of F-DOPA methodology, which is now extended to human subjects after initial work with primates. Using primate model, studies are continuing on metabolic effects of cocaine and fluphenazine. Finally, the Section is continuing its mission to train other research groups in the use of PET imaging technology and data analysis.

Objectives:

The major areas of effort by the seStion has been the development of new methods to the study of normal and abnormal physiology by positron emission tomography and the application of these methods to the study of neuropsychiatric disorders.

Major Findings:

The major focus of the Section has been on the application of Positron Emission Tomagraphy (PET) to the understanding of Attention-Deficit Hyperactivity Disorder (ADHD) and early onset childhood schizophrenia in adult and teenage subjects. During the past year, the following research projects were undertaken by members of the section:

A) PET study of the acute effects of dextroamphetamine and methylphenidate on cerebral metabolism in adults with ADHD was completed by Dr. Matochik and Dr. Zametkin. Both stimulants have modest metabolic effects but produce positive behavioral improvement. Results of the study have been submitted for publication with a new analysis of ROI data using effect size statistics by Dr. Matochik.

B) A long-term PET study of the chronic effects of stimulant medication has been completed and the data are currently being analyzed. Initial results show that chronic treatment in adults has modest metabolic effects but improved behavior as noted by self-report measures, clinical interviews, and psychological testing.

C) A new method of scanning minors which reduces radiation exposure is being used to measure metabolism in normal teenagers and those with ADHD. The description of the method developed by Dr. Zametkin has been accepted for publication by the Archives of General Psychiatry. Teenage schizophrenics are currently being scanned using the new methodology in cooperation with Dr. Gordon of the Child Psychiatry Branch, NIMH. Comparison of metabolism between normal, ADHD and schizophrenic teenagers will provide information that is clinically relevant. Information about developmental differences in metabolic rate between minors and adults is also being obtained.

D) Patients with Generalized Resistance to Thyroid Hormone (GRTH) manifest many of the symptoms of ADHD. Because GRTH has a defined genetic defect, study of this population may provide a unique way to investigate ADHD on the molecular level. Studies in cooperation with Dr. Hauser and Dr. Weintraub of the Molecular and Cellular Endocrinology Branch, NIDDK has shown that: 1) ADHD is highly associated with GRTH in the NIDDK study group and 2) that attention and cerebral metabolism appears to be altered compared to matched controls using PET. The results of the initial study have been sent for publication.

E) In cooperation with Dr. Sunderland of the Unit on Geriatric Psychopharmacology, NIMH, we are using the Double-FDG technique to assess the effectiveness of scopolamine, a cholinergic antagonist, in modeling memory impairment in Alzheimer's Disease. The initial results show altered glucose metabolism in areas of the brain related to memory processes.

F) Dr. Rumsey from the Child Psychiatry Branch, NIMH in cooperation with our Section has completed blood flow studies in persons with dyslexia, one study has been published in the past year which found activation of the left temporoparietal cortex to be task-dependent. A second manuscript is in preparation.

G) In collaboration Dr. Andreason of the Laboratory of Clinical Studies, NIAAA, the neurobiological correlates of mCPP are currently being studied by PET after IV mCPP challenge in early-onset alcoholics. Preliminary results indicate metabolic increases in the cingulate, temporal, and orbital frontal regions.

H) One of the major responsibilities of the Section on Clinical Brain Imaging, in addition to PET studies of psychiatric disorders, is to train and assist other research groups in employing PET imaging technology and data analysis in their research programs. Our group has assisted Dr. Schmidt of the Clinical Pharmacology Section, NIMH in employing the Double-FDG technique to evaluate the metabolic effects of Idazoxan in clinically depressed and normal subjects.

In collaboration with Dr. Elkashef from the Neuropsychiatry Branch, NIMH, we have begun to successfully adapt and implement in human

subjects (normal controls and schizophrenics), the 18F-DOPA scanning method developed by Drs. Doudet and Cohen in non-human primates. This method, based on administration of 150 mg of carbidopa 30-60 min prior to 18F-DOPA injection and the late infusion of unlabelled amino acids (Travasol), has provided in human subjects, the same results reported in monkeys. The method, by reducing non-specific background in PET images, presents several advantages: 1) it enhances the specific/non-specific contrast and visualization of dopaminergic regions, 2) it increases the sensitivity of 18F-DOPA scans to small changes in the dopamine system, and 3) the delay of amino acid infusion permits the acquisition of valid kinetic data during the early time after tracer injection and construction of Patlak plots to yield an 18F-DOPA influx constant. Metabolite analysis is performed under the supervision of Dr. Doudet and Cathy McLellan.

After performing evaluation of the new synthesis of 18F-DOPA implemented by the Nuclear Medicine Department, Dr. Doudet continued to investigate the use of 18F-DOPA to assess dopaminergic function in cocaine-treated rhesus monkeys. With Dr. Carson of the Nuclear Medicine Department, a mathematical model for analysis of 18F-DOPA scans has been developed.

In non-human primates, Dr. Doudet study of acute and chronic effects of fluphenazine on glucose metabolism has shown that acute doses do not induce metabolic changes. The chronic studies are continuing. With Dr. Carson, Drs. Doudet and Cohen continued the evaluation of cyclofoxy kinetics, Bmax and Kd, with the contiuous infusion method. Preliminary data suggest that the continuous infusion method is sensitive enough to demonstrate abnormalities in opiate binding in animals with various brain lesions.

Publications:

Rumsey JM, Andreason P, Zametkin AJ, Aquino T, King AC, Hamburger SD, Pikus A, Rapoport JL, Cohen RM. Failure to activate the left temporoparietal cortex in dyslexias: An oxygen 15 positron emission tomographic study. Arch Neurol 1992; 49:527-534.

Zametkin AJ, Liebenauer LL, Fitzgerald GA, King AC, Minkunas DV, Herscovitch P, Yamada EM, Cohen RM. Brain metabolism in teenagers with attention deficit hyperactivity disorder. Arch Gen Psychiatry in press.

Doudet DJ, McLellan CA, Carson R, Adams HR, Miyake H, Aigner TG, Finn RT, Cohen RM. Distribution and kinetics of 3-O-Methly-6-[F18] fluoro-L-DOPA imaging in the Rhesus monkey brain. J Cereb Blood Flow Metab 1991; 11:726-734.

McLellan CA, Doudet DJ, Brucke T, Aigner TG, Cohen RM. New rapid analysis method demonstrates differences in 6-[18F] fluoro-L-DOPA plasma input curves with and without carbidopa and in hemi-MPTP lesioned monkeys. Appl Radiat Isot 1991; 42:847-854.

Doudet DJ, McLellan CA, Aigner TG, Wyatt RJ, Cohen RM. Delayed L-phenylalanine infusion allows for simultaneous kinetic analysis and improved evaluation of specific to non-specific 18F-DOPA uptake in brain. J Nucl Med in press.

Doudet DJ, Aigner TG, McLellan CA, Cohen RM. 18F-Dopa PET scans: Interpretation and biological correlates in non-human primates. Psychiatry Research in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 MH 02296-07 LCM

PERIOD COVERED
October 1, 1991 to September 30 1992

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,*
PI: C. C. Chiueh Pharmacologist LCM, NIMH

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Cerebral Metabolism

SECTION
Section on Clinical Brain Imaging

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	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.)*
This project is being terminated. Dr. Chiueh has moved to the Laboratory of Clinical Science, NIMH.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00931-19 LGCB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Characteristics and Regulation of S-Adenosylhomocysteine Hydrolase

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

PI:	R.R. Aksamit	Research Chemist	LGCB, NIMH
	P.S. Backlund,Jr.	Research Chemist	LGCB, NIMH
	G.L. Cantoni	Chief	LGCB, NIMH
Others:	M. Fujioka	Toyama Medical & Pharmaceutical University,	Toyama,JN
	G. Gomi	Toyama Medical & Pharmaceutical University,	Toyama,JN
	C.E. Bauer	University of Indiana, Bloomington,	IN
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COOPERATING UNITS *(if any)*

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LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3	1.5	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.)*

S-Adenosylhomocysteine hydrolase is the only enzyme in mammalian cells for the removal of adenosylhomocysteine, the end-product of biological transmethylation reactions. For this reason, the enzyme is critical for the regulation of adenosylmethionine-dependent methylations. We have used several approaches to investigate structure/function relationships of AdoHcy hydrolase. We have cloned and expressed the cDNA for the enzymes from rat liver, D. discoideum, and Rhodobacter capsulatus. The amino acid sequences are highly conserved between species suggesting that much of the sequence is required for enzyme function. Human and bacterial amino acid sequences have 64% sequence identity; to our knowledge this is the highest level of conservation reported between human and prokaryotic enzymes. The putative NAD binding site has been identified by homology to several dehydrogenases and by site-directed mutagenesis of specific amino acids within this region. Investigation of the inactivation of the rat liver enzyme by the site-specific reagent, p-fluorosulfonylbenzoyladenosine, yielded data that support the role of a specific cysteine (cysteine 78) in enzyme function. During inactivation of the enzyme a disulfide between cysteine 78 and cysteine 112 is formed that can be reduced with a thiol to reactivate the enzyme. When cysteine 112 is mutated to alanine, an enzyme with nearly identical kinetic properties is obtained; but upon inactivation by p-fluorosulfonylbenzoyladenosine, cysteine 78 forms a disulfide with cysteine 52. Disulfide formation in the wild-type and mutant enzymes suggests that cysteines 52, 78 and 112 may be near each other in the three dimensional structure of the protein.

We have found a class of organisms that metabolize adenosylhomocysteine by deamination. The Km for AdoHcy is in the micromolar range suggesting that AdoHcy may be one of the normal substrates. The enzyme is inhibited by coformycin and cells treated with coformycin accumulate AdoHcy.

PHS 6040 (Rev. 5/92)

Project Description:

S-Adenosylmethionine (AdoMet) is a key intermediate in biological transmethylation and transalkylation reactions, with hundreds of reactions, each catalyzed by a specific enzyme that utilizes AdoMet as a substrate. 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) is a competitive inhibitor of most reactions in which AdoMet participates. From the results 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 mammals, AdoHcy is metabolized through a single metabolic pathway by AdoHcy hydrolase, an enzyme which catalyzes the reversible hydrolysis of AdoHcy to adenosine and homocysteine. Because of the critical role of AdoHcy hydrolase in the metabolism of AdoHcy and the biological consequences of its inhibition, the enzyme has been under intensive study in this and other laboratories.

1. Structural Determination of AdoHcy Hydrolase.

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 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.

Native rat liver AdoHcy hydrolase is a tetramer of identical 47,300 dalton subunits, and each subunit contains 1 mole of tightly bound NAD. The cDNA sequence has now been cloned from seven species. The amino acid sequences of AdoHcy hydrolase from rat and evolutionally distant species are strikingly similar, suggesting that most of the amino acids are required for enzyme function. Comparison of the rat amino acid sequence with sequences from human, Caenorhabditis elegans, Dictyostelium discoideum, Leishmania donovani, Rhodobacter capsulatus, and parsley show an identity of 97%, 77%, 74%, 74%, 65%, and 64% respectively. To our knowledge the conservation between the human and Rhodobacter sequences in the highest ever reported between a human and bacterial enzyme. The R. capsulatus and parsley sequences differ from the others in that they have an additional sequence of 36 amino acids approximately one third of the distance from the amino terminus. A Drosophila cDNA has also been obtained that codes for a protein whose sequence is 49% identical to the rat liver hydrolase. The sequence indicates that the fly protein is slightly larger than the rat hydrolase because additional amino acids occur at each end. Because of the sequence similarity, it is likely that the fly cDNA codes for AdoHcy hydrolase. We have also shown that fly extracts contain AdoHcy hydrolase activity and plan to express the fly cDNA to confirm that it codes for hydrolase.

E. coli expression vectors have been constructed for the rat liver, Dictyostelium, and Rhodobacter hydrolases. Efficient expression of the rat liver hydrolase has allowed us to examine structure-function relationships of the

enzyme by site-directed mutagenesis. Mutation of key amino acids in a consensus sequence for NAD binding shows that residues 213 to 244 of the rat sequence are involved in NAD binding. Three of the glycines in this sequence are believed to be required for structural flexibility in the binding of the ADP moiety of NAD. Mutation of any one of the residues to a sterically restricted valine produces proteins that do not have catalytic activity, do not bind NAD and no longer have the native quaternary structure. Asp244 within the NAD binding consensus sequence is postulated to interact with the 2'-OH of the ADP moiety of NAD. Mutation of this aspartate to glutamate produces a protein that is inactive when isolated from bacterial extracts, and the purified glu244 mutant protein contains 0.6 moles of NADH/mole of subunits and only 0.05 moles of NAD. The enzyme can be converted to an active enzyme by first removing the NADH and then adding back NAD. The NAD binding constant of the glu244 apoprotein is more than 100-fold less than the binding constant of the wild-type apoprotein indicating the importance of Asp244 in NAD binding.

Previous studies have demonstrated that the inactivation of AdoHcy hydrolase with the compound p-fluorosulfonylbenzoyladenosine (FSBA) was correlated with the formation of a disulfide between cys78 and cys112. To obtain information on the mechanism of inactivation, Cys78 or cys112 was changed to either alanine or serine by site-directed mutagenesis. All four of the mutants were catalytically active. The largest changes in the kinetic parameters occurred when cys78 was mutated suggesting that this cysteine may have a stronger influence on the catalytic reaction than cys112. The pattern of inactivation of the mutants by FSBA (no inactivation with mutants at cys78 and rapid inactivation with mutants at cys112) indicated that FSBA initially reacted with cys78 and the disulfide was subsequently formed by displacement of the modifying reagent by the neighboring cys112.

The sequence of reactions suggested by the above experiments predicts that the inactivated cys112 mutants should have FSBA covalently attached to the protein. However, this was not the case, and sulfhydryl titration experiments showed that approximately two sulfhydryls were lost during the inactivation. This observation together with the finding that activity was regained by incubation with DTT strongly indicates that a disulfide is formed upon FSBA inactivation of cys112 mutants. Available peptide data for the ala112 mutant indicate that a disulfide is formed between cys78 and cys52. The FSBA-inactivation experiments suggest that cys52, cys78 and cys112 are in close proximity in the 3-dimensional structure of the AdoHcy hydrolase.

AdoHcy hydrolase can also be inactivated by incubation with adenosine, and as in the inactivation with FSBA, cys78 mutants are not inactivated by adenosine. The similarity in the FSBA and adenosine inactivation patterns of cys78 and cys112 mutants suggests that cys78 may play an important role in both inactivation reactions. Of interest is the different role of DTT in the two inactivation reactions with rat liver AdoHcy hydrolase. DTT greatly accelerates the inactivation of Adohcy hydrolase by adenosine, but reverses the inactivation by FSBA.

2. AdoHcy Hydrolase Genomic Organization and Expression.

Although AdoHcy hydrolase is present in all cell types that have been examined, it is expressed at a significantly higher level in liver compared to other tissues. Regulatory control occurs at the level of transcription since the amount of mRNA is correlated with the expression of enzyme activity. In order to study the transcriptional control mechanisms of AdoHcy hydrolase expression, the genomic organization of the hydrolase in rat is under study. An examination of rat genomic DNA that can hybridize to the complete cDNA of AdoHcy hydrolase reveals 13 EcoRI fragments that cover approximately 90 kb. Six clones have been analyzed in detail and appear to be different pseudogenes that are approximately 90% homologous to the AdoHcy hydrolase cDNA sequence. At least two of the pseudogenes have introns. Characterization of additional clones will continue with the use of PCR techniques to amplify specific clones which contain intervening sequences in order to eventually find sequences of the AdoHcy hydrolase gene.

3. Biological Effects of Inhibition of AdoHcy Hydrolase.

The development of inhibitors and substrates for AdoHcy hydrolase has allowed us to correlate the inhibition of several biological activities with a decrease in the AdoMet/PurHcy ratio thereby implicating one or more methylases in these processes. Discussions of this work have been detailed in previous reports and in individual project reports Z01 MH 00942 and Z01 MH 02321.

Mutants of Rhodobacter have been obtained that lack AdoHcy hydrolase activity, and these mutants are the first example of a null mutation in AdoHcy hydrolase. The mutants will only grow in media that contain methionine or a methionine precursor, and the mutants have increased levels of AdoHcy and a decreased AdoMet/AdoHcy ratio. The decrease in the AdoMet/AdoHcy ratio would be expected to inhibit methylation, and the synthesis of photopigments whose synthetic pathway includes a methylation is inhibited in the Rhodobacter mutants.

4. Alternative pathways of AdoHcy metabolism.

An individual organism metabolizes AdoHcy by only one of three known pathways: a hydrolase to form adenosine and homocysteine, a nucleosidase to form adenine and ribosylhomocysteine, or a deaminase to form inosinylhomocysteine. Upon screening many organisms for the metabolic fate of AdoHcy, we have identified a class of organisms that deaminates AdoHcy. This reaction was heretofore documented in only one organism, unrelated to the class of organisms that we have identified. The Km for AdoHcy is in the micromolar range suggesting that AdoHcy may be one of the normal substrates for the deaminase. The enzyme is inhibited by coformycin and cells treated with coformycin accumulate AdoHcy.

Significance to Biomedical Research and the Program of the Institute:

Studies of AdoHcy hydrolase are important to understanding the regulation and function of biochemical transmethylations, and inhibitors of this enzyme have possible clinical applications in the development of specific inhibitors for certain methylation reactions. Inhibitors of methylation reactions have been shown to affect cell differentiation, leukocyte chemotaxis, and virus

replication. Understanding the structure/function relationships for AdoHcy hydrolase should make it possible to design novel inhibitors and substrates for the enzyme based on a rational understanding of the catalytic site of the enzyme. 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 methylations in neurotransmitter synthesis, these compounds could have important effects on brain function as well.

Proposed Course:

The determination of the sequence of AdoHcy hydrolase from other species, particularly Drosophila, and the study of mutant AdoHcy hydrolase proteins, generated by site-directed mutagenesis, will continue in order to correlate structure and function. Efforts are being made to crystallize the enzyme in order to determine its three dimensional structure. To further our understanding of the intracellular role of AdoHcy, we plan to initiate studies on the functional properties of two enzymes involved in an alternative metabolic pathway for AdoHcy that occurs in the Enterobacteriaceae.

Publications:

Sganga MW, Aksamit RR, Cantoni GL, Bauer CE. Mutational and nucleotide sequence analysis of S-adenosyl-L-homocysteine hydrolase from Rhodobacter capsulatus, Proc Natl Acad Sci USA 1992; in press.

Mudd SH. Homocystinuria. In: Wyngaarden JB, Smith LH Jr., Bennett JC, eds. Cecil Textbook of Medicine, 19th. Philadelphia: WB Saunders, 1992;1106-07.

Blom HJ, Davidson AJ, Finkelstein JD, Luder AS, Bernardini I, Martin JJ, Tangerman A, Trijbels FJM, Mudd SH, Goodman SI, Gahl WA. Persistent hypermethioninemia with dominant inheritance, J Inher Metab Dis 1992;15:188-97.

Mudd SH, Levy HL, Skovby F. Disorders of transsulfuration. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The Metabolic Basis of Inherited Disease, 7th. New York: McGraw-Hill, Inc., 1992; in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 00942-11 LGCB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Biochemical Reactions in Mammalian Cell Chemotaxis

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

PI:	P.S. Backlund, Jr.	Research Chemist	LGCB, NIMH
	R.R. Aksamit	Research Chemist	LGCB, NIMH
	G.L. Cantoni	Chief	LGCB, NIMH
Others:	A. Spiegel	Scientific Director	IRP, NIDDK

COOPERATING UNITS *(if any)*

Molecular Pathophysiology Branch, NIDDK

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	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 mechanism of leukocyte chemotaxis is being studied as a model for signal transduction by a G-protein coupled receptor. Studies in this laboratory have demonstrated a role for a methylation reaction in leukocyte chemotaxis and in other important biological responses. Protein carboxyl methylation is being studied as a posttranslational modification found in a number of membrane proteins possibly involved in signal transduction.

A. A role for the G-protein G_i-2 in the chemotaxis of macrophage and neutrophils has been demonstrated by this and other laboratories. The role of methylation in membrane attachment and function of G-proteins was studied. We have demonstrated carboxyl methylation and processing of the G-protein γ-subunit in brain. The G-protein γ-subunit contains a C-terminal Cys-Axx-Axx-Xxx sequence, which is modified by a multi-step process including isoprenylation and methylation. We have demonstrated that isoprenylation by itself was not sufficient for membrane attachment of an otherwise soluble protein.

B. We have identified a GTP-dependent carboxyl methylation of several low-molecular weight GTP-binding proteins. One of the methylated proteins in brain was identified as G25K, and additional methylated proteins were observed in a macrophage cell line. The properties of the reaction indicate that methylation may regulate the association of these proteins with the membrane. By altering the localization of these proteins, methylation may act as a molecular switch to regulate transduction of specific biochemical signals.

Carboxyl methylation may provide a novel target for pharmacologic manipulation of a variety of cell signaling systems controlling cell growth, motility, neurotransmitter release, and cell-cell interactions.

Project Description:

This project is investigating the biochemical steps involved in leukocyte chemotaxis as a model for receptor mediated signal transducing mechanisms. Leukocyte chemotaxis is mediated through specific chemoattractant receptors, which are coupled with one or more guanine nucleotide-binding proteins (G-proteins). Several approaches are being used to investigate the biochemical steps, such as methylations, required for the transduction of signals from the receptor to the final biological responses.

A. The important discovery in this laboratory that chemotaxis by a macrophage cell line is specifically inhibited by 3-deaza-AdoHcy allowed us to assess the significance of certain biochemical reactions in macrophage chemotaxis. We were able to rule out the requirement for the following reactions in chemotaxis: the synthesis of phosphatidylcholine by methylation of phosphatidyl-ethanolamine, the release of arachidonic acid when cells were incubated with an attractant, and methylation of the lysine and arginine residues of protein. However, the synthesis of a small number of proteins, did show the necessary inhibitor specificity for involvement in RAW264 chemotaxis.

B. The role of G-proteins in chemotaxis has been investigated using bacterial toxins, such as cholera toxin or pertussis toxin. In the RAW264 macrophage cell line, chemotaxis is inhibited upon incubation of the cells with either pertussis or cholera toxin. The inhibition of chemotaxis is correlated with the ADP-ribosylation of specific G-proteins, but is not correlated with cAMP levels. Using immunochemical studies, we have shown that Gi-2 is the major pertussis toxin substrate in the RAW264 macrophage cell line, and that under some conditions Gi-2 can also be a substrate for ADP-ribosylation by cholera toxin. It is likely that Gi-2 is the guanine nucleotide binding protein that couples chemoattractant receptors to an effector protein such as phospholipase C or ion channels.

A role for methylation in G-protein function is suggested by our discovery of the methylation the carboxyl terminal of the γ-subunit of Gi. The γ-subunit of the G-proteins have a Cys-Aaa-Aaa-Xaa (cys-aliphatic-aliphatic-any amino acid) carboxyl terminal amino acid sequence, which is modified by a multistep process involving isoprenylation of the cysteine, removal of the C-terminal tripeptide, and methylation of the free α-carboxyl group of the cysteine. The role of isoprenylation and methylation on membrane localization was investigated by constructing mutant Gi α-subunits, which remain soluble due to the lack of the N-terminal myristoylation site. The mutation of the C-terminal tetrapeptide was sufficient to specify the addition of either a farnesyl or geranylgeranyl isoprenyl group to the protein. However, neither isoprenyl modification was sufficient for membrane localization of the protein.

C. A guanine-nucleotide dependent carboxyl-methylation has been discovered in the RAW264 macrophage cell line and in brain. In addition to the heterotrimeric G-proteins, cell membranes contain a number of low molecular weight GTP-binding proteins, in the molecular weight range of 20,000 to 25,000. Methylation of some of these proteins required AdoMet, GTP or GTP-analogs, cell membranes and a cytoplasmic protein fraction. A cytosolic protein required for methylation was purified from brain homogenates and identified as the GTP-binding protein G25K.

GTPγS stimulated the methylation of G25K by increasing the affinity of G25K for the methyltransferase. The methyltransferase for G25K methylation appears to be localized to the membrane. This soluble form of G25K was isolated as a heterodimer with a 28 kDa protein. The 28 kDa protein appears to regulate G25K methylation. Association of G25K with the 28 kDa proteins inhibited the methylation and changed the specificity for different guanine nucleotides.

Significance to Biomedical Research and the Program of the Institute:

The importance of guanine nucleotide binding proteins in receptor mediated function is well documented. The carboxyl methylation we have characterized has properties which suggest that these methylations may regulate the function of some guanine nucleotide-binding membrane proteins. An understanding of the relationships between receptors and transducing G-proteins will provide a basis for pharmacologic manipulation of receptor systems at both the receptor and G-protein level.

Stress-induced neuropeptides are known to modulate several immunological activities. Leukocytes have receptors for beta-endorphin, and other neuropeptides, and human monocytes exhibit chemotaxis to met-enkephalin and beta-endorphin. Identification of the steps involved in chemotaxis could provide a basis for strategies to counteract stress-induced immunological dysfunction.

Mammalian cell chemotaxis is also important in the development of the nervous system, inflammation and wound healing. In addition, chemotaxis is an example of a behavioral response at the cellular level. The mammalian cell line model for chemotaxis that we have developed provides a mammalian system to test concepts developed from bacterial chemotaxis to study the biochemical reactions involved mammalian cell signal transduction.

Proposed Course:

We propose to study the methylation of G proteins further by purification and characterization of the components required for the methylation reaction. Now that a specific methylated protein has been identified, it will be possible to develop substrates to characterize the different steps in the posttranslational processing. Purification and identification of additional methyl-acceptor proteins will determine if these proteins are known members of the small molecular weight GTP-binding membrane protein family, or if these proteins are novel members of this family of GTP-binding proteins. With the purification of specific methylated small molecular weight GTP-binding proteins, the presence of a specific methylesterase activity can be investigated.

Publications:

Backlund, PS, Jr. GTP-Stimulated carboxyl methylation of a soluble from of the GTP-binding protein G25K in brain, J Biol Chem 1992; in press.

Butrynski, JE, Jones, TLZ, Backlund, PS, Jr., Spiegel, AM. Differential isoprenylation of carboxy-terminal mutants of an inhibitory G protein α subunit: Neither farnesylation nor geranylgeranylation is sufficient for membrane attachment, Biochemistry 1992; in press.

Spiegel, AM, Backlund, PS, Jr., Butrynski, JE, Jones, TLZ, Simonds, WF. The G protein connection: molecular basis of membrane association, Trends in Biochem Sci 1991;16:338-41.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02321-07 LGCB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

DNA Methylation and Gene Expression

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

PI:	G.L. Cantoni	Chief	LGCB, NIMH
	A. Razin	Professor, The Hebrew University, Jerusalem, Israel	
Others:	A. Levine	Graduate Student, The Hebrew University, Jerusalem, Israel	
	S. Scarpa	Professor, Laboratorio di Clinica Chirurgica, University of Rome, Italy	

COOPERATING UNITS *(if any)*

Department of Cellular Biochemistry, the Hebrew University, Hadassah Medical School, Jerusalem, Israel; Laboratorio di Clinica Chirurgica, University of Rome, Italy

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	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 vertebrates an inverse relationship between gene expression and DNA methylation is now well established but its mechanism is incompletely understood. Housekeeping genes, which are expressed in every cell are characterized by relatively high densities of unmethylated CpG islands in the promoter region; whenever these CpG residues become methylated the genes become transcriptionally inert. By contrast in tissue specific genes that are normally repressed, Cpg sequences are methylated but become hypomethylated where, or when, the specific genes are expressed. It is noteworthy that the effect of promoter methylation on gene repression can be best demonstrated in vivo. We have investigated two aspects of this multifaceted problem: a) the biochemical mechanism underlying conversion of mCpG residues to CpG residues, and b) the mechanism linking promoter methylation and inhibition of gene expression.

Conversion of mCpG to CpG by removal of the methyl group from methyl cytidine is biochemically impossible as this reaction would involve cleavage of a C-C bond; in order to account for the loss of mCpG during gene expression two different models have been proposed: i) we and others have shown that hypomethylation occurring concomitantly with cell duplication can be explained by inhibition of maintenance methylase through two cycles of DNA replication ii) in the absence of DNA duplication hypomethylation involves replacement of a large fraction of mC in mCpG sequences with cytidine as first demonstrated by Cantoni, Razin and collaborators.

The mechanism by which the presence of methyl groups at the promoter region inhibit gene activity has been pursued by use of engineered constructs in transient transfections. The most effective suppression was observed when methylation was in the pre-initiation domain. Our recent results support earlier suggestion that a mediator protein is involved in the mechanism of promoter inhibition.

Project Description:

One characteristic feature of vertebrate and plant DNA is the presence of a rare nucleotide, 5-methyl-cytidine, that is found exclusively within the dinucleotide 5-mCpG. 5-methylcytidine is formed by postreplicational and irreversible transfer of the methyl group of AdoMet to CpG residues. Methylation of genomic DNA is widespread in the animal kingdom and reaches its highest level in vertebrates where almost 90% of the CpGs may be methylated.

The biological significance of DNA methylation has been investigated extensively in the past decade. It was established first of all, that at any one time specific CpG sites are either methylated or unmethylated on both DNA strands and that the pattern of DNA methylation is stably maintained over many cell generations and clonally inherited through the activity of a maintenance methylase.

An exploration of the distribution of mCpG in the vertebrate genome suggested the existence of an inverse correlation between DNA methylation and gene expression: the promoter of housekeeping genes, which are expressed in every cell, display relatively high densities of unmethylated CpG islands that can under some circumstances become methylated. Little is known about the factors that trigger de novo methylation of these unmethylated sequences, but it is clear that methylation renders these genes transcriptionally inert. By contrast the CpG sequences found in tissue specific genes, that by definition are silent, are methylated but these become hypomethylated where, or when these genes are expressed.

While the inverse relationship between DNA methylation and gene expression is well established its mechanism is still obscure. Three mechanisms have been suggested: promoter methylation might i) hinder binding of transcription factors directly thereby preventing gene expression; ii) act indirectly through a mediator protein capable of binding specifically to methylated sites in the promoter and thereby preventing the formation of transcription complex; iii) affect the organization of the chromatin structure. The recent identification of proteins capable of binding specifically to methylated DNA and other lines of evidence favor the indirect mechanism.

A) Changing the methylation pattern in DNA. The existence of biochemical mechanisms for the conversion of mCpG to CpG sequences follows directly from the data reviewed above. As noted above the transfer of the methyl group of AdoMet to CpG residues is irreversible; direct removal of the methyl group from carbon 5 of cytidine, a reaction that would involve cleavage of a C-C bond, is most unlikely and without precedent.

During differentiation changes in the pattern of DNA methylation with a decrease in the fraction of mCpG doublets has been demonstrated both in dividing and non-dividing cells and two different models have been proposed to explain these observations. In dividing cells the replacement of mCpGs with CpGs could result from the inhibition of maintenance methylase through two cycles of DNA replication; in fact it has been shown by us and by others that an increase in the intracellular level of methylase inhibitors such as AdoHcy or its congener, DZAHcy, results in a decrease of the amount of mCpG in DNA. Others have shown

that 5-azacytidine after incorporation into DNA is capable of inhibiting specifically the activity of maintenance methylase, thereby leading to the reactivation of genes that were previously methylated and repressed. However, it has not yet been determined whether inhibition of maintenance methylase during DNA replication is physiologically relevant since neither fluctuations in the activity of AdoHcy hydrolase nor the natural presence of 5-azacytidine-like nucleosides has yet been demonstrated.

In cell culture the differentiation of myoblasts into myotubes requires two to three cycles of DNA replication and is stimulated by addition of 3-DZA + HcY. This system may therefore be used to explore if the level of AdoHcyase is altered during differentiation. S. Scarpa at the University of Rome, Italy, in collaboration with G.L. Cantoni is using the cDNA coding for rat liver AdoHcyase as a probe of the expression of the AdoHcyase gene during differentiation.

In the absence of DNA replication, a different and novel model has been proposed and experimentally verified in collaboration with A. Razin and his colleagues in Jerusalem. We have shown that one of the early events in the differentiation of FELC is the replacement of a large fraction of mC in mCpG sequences with cytidine. The incorporation of cytidine residues in non replicating DNA, catalyzed by 5-mC replacase, is highly specific. We also concluded that 5-mC replacase may be present in an inactive form before differentiation. In collaboration with A. Levine and A. Razin we have begun a series of experiments designed to identify and isolate the enzyme(s) involved in the replacement of C for mC in CpGs during the early phases of erythroid differentiation.

B) Promoter methylation and transcriptional inactivity. To explore the mechanism whereby DNA methylation inhibits gene expression we transfected into Ltk- or F9 cells constructs of human growth hormone gene fused to methylated or unmethylated mouse metallothionein promoter or the HSV thymidine kinase promoter. In this system the human growth hormone gene, or thymidine kinase, serve as reporters of the transcriptional activity of the promoters. Expression of the reporter gene was greatly reduced when constructs in which all CpGs had been converted to mCpGs by M.SssI methylase were transfected in Ltk- or F9 cells. However transfection of constructs in which only a fraction (10%) of the available CpGs had been methylated yielded different responses in the two cell types: in Ltk- cells gene expression was greatly reduced while in F9 cells the activity of the reported gene was not affected at all. These data suggest that the inhibition of gene expression by promoter methylation might be due to a protein factor capable of binding to methylated DNA and that this factor would be present in Ltk- cells but missing in F9 cells.

C) Location and density of methyl groups. In order to explore the relationship between location and density of methyl groups and gene repression it would be advantageous to use promoters in which the position and number of methyl groups along the promoter region can be controlled unambiguously. Therefore we have transiently transfected into Ltk- and other cells, a series of methylatable constructs in which chemically synthesized oligonucleotides have been inserted into various positions of the promoter region. These experiments clearly showed that the presence of methylated CpG sequences in close proximity to the TATA box

was most effective in suppressing the activity of reporter genes. It may also be concluded from our experiments that the formation of an initiation complex, a key step in gene expression, requires the participation of a protein with special affinity to methylated DNA.

Proposed Course:

We plan to study further the biochemical mechanisms underlying the changes in the pattern of DNA methylation during differentiation in dividing and non-dividing cells as well as the relationship between promoter methylation and repression of gene expression. In both cases we postulate that these phenomena are due to protein factors. If this hypothesis is correct it should be possible to identify and characterize these proteins by standard purification techniques provided enough material can be obtained.

Additional support for this project continues to be provided by a grant to A.Razin and G.L. Cantoni from the United States-Israel Binational Science Foundation.

Publications:

Levine A, Cantoni GL, Razin A. Inhibition of promoter activity by methylation: Possible involvement of protein mediators, Proc Natl Sci USA 1991;88:6515-18.

Levine A, Cantoni GL, Razin A. Methylation in the preinitiation domain suppresses gene transcription by an indirect mechanism, Proc Natl Sci USA 1992;89, in press.

PROJECT NUMBER

Z01 MH 02162-04 LGCB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

S-Adenosylmethionine and Affective Disorders

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

PI:	G.L. Cantoni	Chief	LGCB, NIMH
Others:	A. Merta	Visiting Associate	LGCB, NIMH
	E.I. Ginns	Chief	NSB, NIMH

COOPERATING UNITS *(if any)*

Clinical Neuroscience Branch, NIMH

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1	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.)*

S-Adenosyl-L-methionine (AdoMet) is a safe and probably effective antidepressant agent in certain forms of clinical depression. A new hypothesis is offered to account for the mechanism of action of S-adenosylmethionine in such illness, based upon the known biochemistry of this compound, and upon current knowledge of clinical and genetic aspects of affective disorders. We postulate that at least some major mood disorders are due to abnormalities affecting the AdoMet-dependent methylation of a substance in the central nervous system. For convenience and without prejudging the chemical structure of this substance, we term it for the moment "barinine." The model requires that barinine be subject to AdoMet-dependent methylation and that methylbarinine be subject to metabolic demethylation to regenerate the original barinine. Methylbarinine should be mood elevating, whereas barinine itself should not be. Depression is a result of abnormalities lowering the normal steady-state concentration of methylbarinine, while mania results from an abnormal elevation of methylbarinine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 01037-23 LMB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Receptor-Mediated Transport of Proteins and Viruses Across Cell Membranes.

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

P.I.:	D. M. Neville, Jr.	Chief, Sec. on Biophys. Chem.	LMB, NIMH
Others:	M. Akeson	Visiting Scientist	LMB, NIMH
	K. Shailubhai	Staff Fellow	LMB, NIMH
	K. Rigaut	Staff 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:	PROFESSIONAL:	OTHER:
6	4	2

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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.)

The general aim of this project is to elucidate mechanisms by which proteins and viruses enter cells by receptor-mediated transport processes and translocate across the lipid bilayer to cytosol and nuclear compartments. Molecular signals which lead to unique vesicle trafficking are studied. Basic knowledge from these processes is used to design targeted drug delivery systems such as immunotoxins.

Anti-human CD3 immunotoxins constructed with CRM9, a binding site mutant of diphtheria toxin, eliminate 80% of established tumors of human T cell leukemia in a nude mouse xenograft system. The cell killing power of the immunotoxin, when delivered at 1/2 the guinea pig minimum lethal dose, is equivalent to 500-600cGy from a ^{137}Cs source and produces a 3 log kill of tumor cells. The therapeutic margin appears promising for clinical application which might include treatments for AIDS, autoimmune diseases and graft-versus-host-disease following bone marrow transplantation. Pre-clinical trials in non-human primates are now in progress.

The diphtheria toxin receptor has been identified as belonging to the class of plasma membrane endosome recycling receptors.

The plasma membrane potential sensitive step in vesicular stomatitis virus infection involves an early cytosolic pre-transcription step or transcription itself.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

The general aim of this project is to elucidate mechanisms by which proteins and viruses enter cells by receptor-mediated transport processes and translocate across the lipid bilayer to the cytosol and nuclear compartments. Molecular signals which lead to unique vesicle trafficking are studied. Basic knowledge from these processes is used to design targeted drug delivery systems such as immunotoxins.

Major Findings:

Anti-human CD3 immunotoxins constructed with CRM9, a binding site mutant of diphtheria toxin, and utilizing an acid-labile crosslinking agent, eliminate established tumors of human T cell leukemia in a nude mouse xenograft system. Using an immunotoxin dose set at 1/2 of the minimum lethal dose for CRM9 in sensitive animals, complete regressions were achieved in 80% of the tumor bearing animals. In the past these levels of response in this type of animal model has been predictive of partial responses and some complete responses in clinical trials.

We have now constructed the rhesus analog of this anti-CD3-CRM9 immunotoxin and have synthesized sufficient immunotoxin for a pre-clinical trial of T cell ablation and immunosuppression in rhesus monkeys. The trial is currently in progress.

The diphtheria toxin receptor has been identified as belonging to the class of plasma membrane recycling receptors. In addition this receptor exhibits a polarized distribution on a variety of polarized epithelial cells.

The voltage sensitive step in vesicular stomatitis virus infection has been found, on the basis of fluorescent staining assays, to be just distal to the viral nucleocapsid cytosolic translocation step. This places the voltage sensitive step at an early cytosolic pre-transcription processing event or at primary transcription per se.

Significance to Biomedical Research and the Program of the Institute:

The ability of anti-CD3-CRM9 based immunotoxins constructed to reduce pan T cell populations *in vivo* provides an immunosuppressive therapy which hopefully will be free of toxic side effects which limit current immunosuppresive regimes. Possible uses are in treating a variety of autoimmune diseases, the treatment of graft-versus-host-disease, and in reducing the burden of retroviral infected T cells in AIDS.

Elucidation of the diphtheria toxin routing pathway will permit identification of other cell surface epitopes which follow this pathway permitting their use as effective targets for anti-CRM9 immunotoxins. Thus the utility of these highly effective immunotoxins will be broadened beyond their current application to pan T cells.

Knowledge of the early steps in enveloped viral infections is currently meager. Detailed knowledge in this area should permit the rational design of anti-viral drugs.

Proposed Course:

The *in vivo* anti-human CD3 immunotoxin studies on T cell ablation done initially in a nude mouse xenograft system has been shifted to a simian model and these studies will be pursued in depth. Thus it will be possible to see if the promising results obtained with the xenograft tumor system are transferable to an endogenous circulating T cell system. A simian model of

autoimmune diseases (rheumatoid arthritis) is available and several simian model of AIDS are available.

The intracellular routing of the diphtheria toxin receptor will be elucidated. Assays will be designed to test which surface epitopes of any test cell route in parallel with the receptor. CRM9 based immunotoxins will be developed towards a variety of cell types for therapeutic purposes.

A variety of studies will be initiated to identify the exact step in vesicular stomatitis virus trafficking which is membrane potential sensitive. A particular interest is whether this step involves an unknown pre-transcriptional nucleocapsid processing step or whether transcription itself is under the influence of the plasma membrane potential, either directly or through some intermediate.

Publications:

Akeson, M., Scharff, J., Sharp, C.M. and Neville, D. M., Jr.: Evidence that plasma membrane electrical potential is require for vesicular stomatitis virus infection of MDCK cells: A study using fluorescence measurements through polycarbonate supports. Membrane Biology 125: 81-91, 1992.

Neville, D. M., Jr., Scharff, J. and Srinivasachar, K: In Vivo T Cell Ablation by a Holo-Immunotoxin Directed at Human CD3. Proc. Nat. Acad. Sci. USA 89: 2585-2589, 1992.

P. Bogner, P. Skehan, S. Kenney, E. Sainz, M. A. Akeson and S. J. Friedman: Stabilization of intercellular contacts in MDCK cells during Ca++ deprivation: selective effects of monocarboxylic acids on desmosomes. Journal of Cell Science (in press).

Neville, D. M., Jr., Scharff, J. and Srinivasachar, K.: Anti-T Cell Immunotoxins. A Look At Post-Endocytotic Receptor-Mediated Routing. J. of Controlled Release: (In press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02228-08 LMB

PERIOD COVERED
October 1, 1991 to September 30, 1992

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)*

P.I.:	Howard A. Nash	Chief	LMB, NIMH
Others:	Boris Leibovitch	Visiting Scientist	LMB, NIMH

COOPERATING UNITS *(if any)*

Howard Hughes Medical Institute, Bethesda, MD

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Molecular Genetics

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	0.75	1.75

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.)*

The potency of volatile anesthetics and n-alkanes as agents of general anesthesia has been determined in Drosophila melanogaster. The correlation of potency with hydrophobicity and the absolute concentration required to achieve anesthetic effects are similar to those reported in higher organisms. Thus, Drosophila has been confirmed as a suitable model system for study of the mechanism of general anesthesia. Several mutants of the fruit fly that are altered in their response to halothane have been studied further. One mutant loses its anesthetic resistance upon decapitation while three other mutants retain resistance. This analysis highlights the anatomic complexity of the anesthetic response. In another study, wild-type and mutant intact flies have been assessed in a time-independent assay of the escape from a noxious stimulus. This tail-flick assay for fruit flies shows that each mutant locus differentially affects the response to different anesthetics. The result argues against the hypothesis that all anesthetics interact in identical manner with the anesthetic target.

GENETIC NEUROBIOLOGY OF DROSOPHILA

Objectives

The nervous system of Drosophila contains tens of thousands of nerve cells organized into diverse structures that control an impressive array of complex behaviors. The short generation time and small genome of Drosophila have expedited a genetic analysis of many neural phenomena; single locus mutations that alter specific aspects of memory, courtship and circadian behavior are known. This project seeks to apply similar genetic techniques to an analysis of the action of general anesthetics. In the long term, we want to use mutations to identify the critical target(s) whose alteration produces the anesthetic state and we want to use general anesthetics as a tool to find genes that govern neural function in the fruit fly. In the past year, our objectives were to quantitatively assess the claim that Drosophila is a suitable model system for anesthesia in higher organisms and to probe the pharmacodynamics of a novel class of anesthetics. We have also sought to extend our characterization of mutants isolated as resistant to halothane so as to challenge the classical unitary hypothesis of anesthetic action.

Major Findings and Methods Employed

In higher organisms, it has been repeatedly observed that there is a remarkable correlation between the dose of an agent required to produce anesthesia and the hydrophobicity of that agent, as measured by its solubility in olive oil. This correlation is not only valuable in predicting the potency of new agents, it provides the lone insight to the nature of the anesthetic target, i.e. it must be hydrophobic. Although our laboratory has already shown that Drosophila responds to many clinical anesthetics, the observations were limited to assessment of motor control in response to a fixed concentration of each agent. We have now measured dose-response curves for nine volatile anesthetic liquids. Anesthetics were introduced into a glass chamber, allowed to evaporate, and sampled by gas chromatography. Flies were then introduced into the chamber and observed for loss of their natural geotaxis. Each chamber provides a time course of the response to a measured concentration of anesthetics; from several such chambers we calculate an ED50, the dose that anesthetizes half of the flies, as a function of time. We find that for each anesthetic, ED50 asymptotes to a fixed value in the course of one to three hours, thus establishing a time-independent measure of potency for the nine anesthetics. We have also used gas chromatography and glass chambers to measure the way these agents partition between air and olive oil. We find that, just as for higher organisms, there is an excellent correlation in Drosophila between hydrophobicity and potency. Moreover, the absolute concentration of volatile agents required to produce anesthetic effects is quite comparable to that seen with mammals. These results strongly support the contention that the anesthetic target has been conserved throughout evolution.

We have previously shown that n-alkanes of chain length less than ten can act as anesthetics in Drosophila. We have now measured the olive oil/gas partition coefficients for these alkanes and find that there is a significant disparity between the observed potency and that predicted by their oil solubility; the alkanes are less potent than expected and the discrepancy increases with increasing chain length. We have shown that flies recover from alkanes as rapidly as from conventional anesthetics. This argues against artifacts introduced by rapid metabolism of alkanes. We conclude that alkanes are unusual anesthetics, ones whose target is not well-modeled by olive oil. The same conclusion has been independently reached by Eger and colleagues in a recent study of alkane anesthesia in rats. These findings confirm our contention that the anesthetic target has been conserved.

Our previously isolated mutants that change the response of Drosophila to halothane should provide clues about the components of the nervous system that are affected by anesthetics. One

way to ask about the nature of the changed component is to determine its anatomical sphere of action, i.e. which tissue of Drosophila needs to be mutant in order to produce a mutant phenotype. We have begun this search by determining the role of the head. Like other insects, Drosophila survives for extended periods after decapitation. We therefore cut the connections between the head and the body, allowed the flies to stabilize overnight, and tested them for their responsiveness to anesthesia. As before, our assay was a column equipped with nylon mesh baffles that act as attractive resting places for flies in the awake state but poorly retard flies rendered uncoordinated by anesthetics. We first characterized the response of wild-type flies. We found that, in the absence of anesthetics, headless flies dwelled in the column about as well as intact flies and that both kinds of flies elute upon exposure to 0.5% halothane. Three of our mutants, har38, har85, and Har63, retained their resistant phenotype in the headless state, i.e. they eluted slower than their decapitated wild-type counterparts. On the other hand, headless flies of the Har56 line were as sensitive to halothane as were headless wild-type flies. We conclude that the latter mutation affects a component that resides in the head or depends upon a signal from the head while the other alleles affect autonomous neural elements in the body. Thus, the decapitation assay has distinguished at least two anatomical sites of gene action.

Using the column assay, we had previously determined the response of wild-type and mutant flies to several anesthetics other than halothane. We found that each of the three loci represented by these four mutants show a pattern of response that is different from wild-type flies and different from each other. We have now tested our mutant flies using a second assay. We treated groups of 10 flies to a given dose of anesthetic and then exposed each fly in the group to an intense beam of light (delivered by a conventional tail-flick apparatus). Failure to move purposely in response to this noxious heat stimulus within six seconds is counted as evidence of the anesthetized state. The experiment is continued until the proportion of non-responding flies is constant; then the dose of anesthetic is increased and the process repeated. From the data we calculate a time-independent ED50. As before, each mutant locus differs from wild-type and from each other. The pattern of responses is complex. For example, compared to wild-type flies, Har63 flies are strongly resistant to enflurane and methoxy-flurane, weakly resistant to isoflurane, hypersensitive to chloroform, halothane and trichloroethylene, and similar to wild-type in their response to diethylether. On two grounds these data indicate that the mutations alter pharmacodynamics and not just pharmacokinetic aspects of anesthesia. First, an alteration in ED50 is seen in the steady state, so models that invoke altered rates of passive entry and/or distribution of anesthetics are eliminated. Second, in several cases a mutant line that is altered in response to an anesthetic in the tail-flick assay is not altered in the column assay. This eliminates models that postulate differences in overall metabolism of the anesthetic. The new data also show that the mutations affect the quintessential behavior that is typical of anesthesia in higher organisms, i.e. the perception of and/or the response to pain. Because the new data indicate a pharmacodynamic modification in a fundamental anesthetic trait, they complete the disproof of the classical view of anesthesia which suggests that all volatile anesthetics have identical effects at the anesthetic target. Our discovery of mutants that significantly change the effectiveness of different anesthetics almost certainly means that neural components normally distinguish between anesthetics and these distinctions are heightened by mutation.

Significance to Biomedical Research on the Program of the Institute

The altered state of consciousness that is induced by general anesthetics is not reproduced by individual pharmacological agents whose mechanism of action is clearly understood. It has therefore been difficult to decide whether volatile anesthetic liquids produce their clinically valuable effects by altering a single, previously unidentified neural component or by altering several components, each of which may be already identified. Our work on the genetics of the anesthesia response in Drosophila provides an avenue to address this question. Our

demonstration that the potency of a broad range of conventional anesthetics and a series of n-alkanes shows the same correlation with olive oil solubility in Drosophila as is seen with these agents in higher organisms argues strongly that the anesthetic target is conserved. Thus, our analysis of mutant flies whose anesthetic sensitivity is altered should be applicable to the clinical setting. Our finding that mutations can differentially affect anesthetic sensitivity provides strong evidence that, despite their nearly uniform concentration in a hydrophobic phase, different anesthetics interact differently with the anesthetic target. Although we do not know whether different anesthetics work at different targets or have a differential effectiveness of the same target, our study points the way to answer this question.

Proposed Course

The study of the measurement of anesthetic potency in Drosophila has been prepared for publication. The discovery that alkanes have an anomalous potency identifies these agents as compounds whose interaction with the anesthetic target is unusual. Therefore, they will be used to distinguish amongst existing mutants and used as a basis for isolating new mutants. In this way, we should be able to learn whether the target for alkane anesthesia is distinct or overlaps with the target of conventional anesthetics. The demonstration that different mutant loci differentially affect sensitivity to several anesthetics needs to be extended. Specifically, we need to know if mutant flies show an overall correlation between potency and hydrophobicity despite their altered response. Tail-flick assays with compounds at the low and high end of the hydrophobicity spectrum e.g. desflurane and methoxyflurane, should be informative. Finally, we need to know how our existing mutants interact with other genetic loci that affect known neural components and we need to expand our collection of anesthesia mutants so as to provide a clearer view of the genetic complexity of the anesthetic response.

Publications

None

APPENDIX TO ANNUAL REPORT SUMMARY

I. Research Highlights

- Dose - response curves have confirmed the fruit fly as a suitable model for study of the mechanism of general anesthesia.
- The response of mutant flies to different anesthetics has disproved the unitary theory of anesthetic action.

II. Lab/Branch Plans

- A search will be undertaken for mutants of Drosophila where insensitivity to octane anesthesia approaches that of conventional anesthetics.
- The autosomes of Drosophila will be screened for loci that can mutate to halothan resistance.
- The interaction between genes that govern brain neural elements and those that control the anesthetic response will be explored.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 01035-23 LMB

PERIOD COVERED
October 1, 1991 to September 30, 1992

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

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

P.I.:	H. A. Nash	Chief	LMB, NIMH
Others:	S. D. Goodman	Staff Fellow	LMB, NIMH
	A. E. Granston	Staff Fellow	LMB, NIMH
	A. M. Segall	Guest Research	LMB, NIMH
	G. V. Shpakovski	Visiting Fellow	LMB, NIMH
	A. B. Burgin	Guest Researcher	LMB, NIMH
	S. Yang	Visiting Fellow	LMB, NIMH

COOPERATING UNITS *(if any)*
Laboratory of Biochemical Pharmacology, NIDDK
Laboratory of Chemical Physics, NIDDK
Center fopr Advanced Research in Biotechnology, Gaitherburg, MD

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Biophysical Chemistry

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
7.25	6.25	1.0

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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.)*

Mechanistic aspects of site-specific recombination were explored. To distinguish between models for the chemical step of recombination, alterations were made in nucleophiles that could potentially attack DNA. The evidence pointed to a symmetric mechanism for strand exchange in which a nucleophile from different protomers of a topoisomerase attack each partner in the recombination event. In a second study, the functional connection between two related proteins that cause DNA deformation was established and explored. The structural basis for the functional connection was demonstrated by showing that both proteins, which differ in their degree of DNA binding specificity, could assist the formation of the same nucleoprotein complex.

A search for homologs of site-specific recombinases was refined. The sequence of candidate clones from S. pombe showed no significant homology to the target family and were judged to be coincidental matches to the probe. However, the clones did contain apparent homologs of the S. cerevisiae genes ARG4, ILV5 RPB6.

PHS 6040 (Rev. 1/84) GPO 914-918

THE PROCESS OF LYSOGENY

Objectives

This project explores the way a programmed rearrangement of DNA is achieved in nature. We focus on the reaction that integrates the DNA of a bacterial virus, phage lambda, into a specific locus on the genome of its bacterial host, E. coli. We want to understand how interactions between specific proteins and their DNA targets accomplish a precise and efficient joining of two pieces of DNA. In the past year our research has questioned the chemical nature of the events that break and rejoin DNA in the two partners, the functional and structural relationships between a pair of recombination accessory proteins and the extent to which recombination systems similar to those of lambda integration can be found in diverse organisms.

Major Findings and Methods Employed.

Integrative recombination involves two proteins: a virus-encoded recombinase, Int, and a host-encoded accessory protein, Integration Host Factor (IHF). Int protein is a topoisomerase, i.e. it can use a nucleophile from one of its tyrosine residues to attack the phosphodiester backbone of DNA and simultaneously create a covalent enzyme-DNA bond. There is much evidence that such cleavage is involved in the initial step of recombination at the bacterial recombination locus, attB. However, it is not clear which nucleophile attacks the corresponding strand of the attP partner. While a second protomer of Int could provide this nucleophile, it is equally plausible to imagine that the nucleophile comes from the DNA fragment of that attB that is created by the first attack. We have distinguished between these alternatives by manipulating the enzyme and its DNA substrates. For example, we artificially created a DNA-derived nucleophile by nicking attB and asked whether this relieved the requirement for an enzymic tyrosine to catalyze strand exchange. The negative results suggested that attack of attP is not by nucleophile from attB. This hypothesis was supported by experiments in which attB was artificially cleaved in half so as to remove any potential source of an attacking DNA nucleophile. This artificial substrate was demonstrated to still be a competent partner for recombination with attP. We conclude that there is symmetry in the mechanism of integrative recombination; like attB, attP is attacked by an enzymic nucleophile.

The strong homology between their amino acid sequences indicates that IHF protein is structurally related to the HU proteins, a family that is widely distributed in procaryotes, mitochondria and chloropalasts. Like IHF, members of the HU family bind and deform DNA, but unlike IHF, HU proteins bind to DNA and with low affinity and with little or no sequence specificity. Previous work from our laboratory has shown that HU cannot replace IHF for integrative recombination but we now have demonstrated that excisive recombination, the site-specific deletion of the integrated viral chromosome, can be promoted by purified HU protein. Moreover, studies with cells mutant for either or both protein confirm that HU can substitute for IHF during in vivo excisive but not integrative recombination. We believe that IHF enhances both integrative and excisive recombination by deforming DNA so as to facilitate construction of precise and compact nucleoprotein arrays. To test whether HU substitutes for IHF in this function or whether it stimulates excisive recombination by a different mechanism, we examined the structure of one of the excisive recombination loci, attL. As judged by polyacrylamide gel electrophoresis experiments, HU can combine with Int to produce complexes on attL that are identical to those made by IHF and Int. Mutation of binding sites within attL shows that complex formation with HU depends on a critical pair of binding sites for Int protein but not on a specific IHF binding site. Our data indicates that HU contributes to the formation of a complex in which Int protein bridges two non-adjacent sites in attL. HU is trapped in this complex and thereby gains the site-specificity which it lacks on its own. The failure of HU to replace IHF in integrative recombination suggests that alternate structures are formed and that

some of these must be too weak to successfully trap HU.

Almost 30 homologs of Int recombinase have been identified by sequence and/or functional similarities. Six of these are found in episomal elements of various yeast species, where they function to regulate the copy number of the elements. To search for homologs in more distant organisms, we designed degenerate sets of oligonucleotides that conform to the conserved portion of the yeast recombinases and used them as PCR probes. Because the conserved residues are clustered, the probes were separated by only a few nucleotides. Thus, the primary PCR signal provided little useful information other than indicating that the genome in question contained suitably spaced sequences that matched our initial set of primers. To identify the genomic sequences highlighted by our probes, we isolated individual clones from a genomic library that produced the desired signal. We focused on the genome of S. pombe because its small genome simplified the search, it is only distantly related to the yeast species known to have an Int-like recombinase, and its abundance of introns and their mechanism of splicing suggest that this organism is more closely related to higher eucaryotes than are other yeasts. By repeated subdivision of an S. pombe genomic library, we identified three different segments of DNA that produced a strong signal with our degenerate primers. Subcloning the 20 to 40 kb inserts from these plasmids ultimately permitted the determination of the responsible DNA sequence. In each case, the match to our primers proved to be accidental and did not arise from an open reading frame that encoded a gene with significant homology to the recombinases. Although the project therefore failed, the DNA sequence from the region did reveal previously unidentified open reading frames. Comparison with genes in the database suggests that these are the S. pombe equivalents of the ARG4, ILV5 and RPB6 genes of S. cerevisiae, genes involved in arginine isoleucine and messenger RNA biosynthesis, respectively.

Significance to Biolomedical Research on the Program of the Institute

Virtually every important biological manipulation of DNA - e.g. replication, transcription, repair of damage, etc. - depends on complex arrays of proteins assembled at specific sites in the genome. Our work on proteins that deform DNA so as to assist the construction of these nucleoprotein complexes should be widely applicable. For example, in animal cells researchers have recently identified a set of DNA binding proteins, the HMG family, that has members that display either non-specific (HMG1 and HMG2) or specific (the testis-determining factor and the lymphoid enhancer factor) DNA binding. Our analysis of the functional relationship between a similar set of bacterial proteins opens the way for understanding how such families apportion their essential tasks.

Understanding the chemistry that underlies biochemical transformations is a fundamental goal of biomedical research. Our demonstration of symmetry in the strand exchange of lambda integrative recombination establishes for the first time a pure topoisomerase mechanism, i.e. one in which nucleophiles from a recombinase attack each partner DNA. The chemical basis of lambda integration is now established, and can be used to analyze the relationship to other kinds of recombination at the most fundamental level.

Proposed Course

The question concerning the role of topoisomerases in strand exchange has been answered. A publication describing this work has been prepared. The methods employed in this study suggest novel extensions that should permit the dissection of the elementary steps in the recombination pathways. Specifically, we will try to modify the chemistry of the attacking nucleophiles so as to render certain strand transfer events irreversible. This should allow us to probe for hitherto fleeting events which may well control the fidelity and the directionality of this recombination.

The dissimilar response of integrative and excisive recombination to substitution of IHF by the non-specific DNA binding protein HU deserves attention. We plan to dissect the relevant recombination loci to see if HU can work at some positions within attP but not others. In addition, the entrapment of HU within attL fixes this protein in one place and enables us to study for the first time the details of interaction between HU protein and a particular DNA target. Comparison with similar footprints of IHF should provide the first critical information on the degree of relatedness of the DNA binding character of these two proteins.

The search for homologs of site-specific recombinases has turned up only false positives. We conclude that the information content in the conserved residues in these enzymes is not sufficiently high to fashion a selective search. The project has been terminated. The genes of S. pombe that were accidentally discovered during this work merit further study and will be pursued independently outside this laboratory.

Publications

M. Cowart, S.J. Benkovic and H.A. Nash: Behavior of a cross-linked attachment site: testing the role of branch migration in site-specific recombination. *J. Mol. Biol.* 220:621-629, 1991.

H.A. Nash and A. E. Granston: Similarity between the DNA-binding domains of IHF protein and TFIID protein. *Cell* 67:1037-1038, 1991.

A.B. Burgin, Jr. and H.A. Nash: Symmetry in the mechanism of lambda integrative recombination. *Proc. Nat. Acad. Sci.* (in press).

S.D. Goodman, S.C. Nicholson and H.A. Nash: Deformation of DNA during site-specific recombination of bacteriophage lambda: replacement of IHF protein by HU protein or sequence-directed bends. *Proc. Nat. Acad. Sci.* (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 00934-20 LMB

PERIOD COVERED: October 1, 1991 to September 30, 1992

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:	T. Bzdega	Visiting Associate	LMB, NIMH
	Y. Wang	Visiting Fellow	LMB, NIMH
	H. Chin	Staff Fellow	LN, NINDS
	W.F. Simonds	Staff Fellow	MPB, NIDDK
	K. Rice	Chief, Laboratory of Medicinal Chemistry	NIDDK
	H. G. Coon	Cell Biologist	LG, NCI
	P. Hargrave	Professor of Ophthalmology	Univ. of Florida
	A. Rius	Lab of Developmental Neurobiology	NICHD

COOPERATING UNITS (if any)

Laboratory of Neurophysiology, NINCDS; Laboratory of Medicinal Chemistry, NIDDK; Laboratory of Genetics, NCI; and Laboratory of Biochemistry, NCI

LAB/BRANCH: Laboratory of Molecular Biology

SECTION: Section on Regulatory Proteins

INSTITUTE AND LOCATION: NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	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.)

Many cell surface receptors that mediate the actions of hormones and neurotransmitters are coupled to the activation or inhibition of intracellular enzymes or of ion channels by GTP-binding regulatory proteins (G proteins). The activated G proteins thereby transmit information about receptor occupancy to the interior of cells. We have, for many years, studied one of these G protein coupled receptors: the opiate receptor. The neuroblastoma x glioma hybrid cell line, NG108-15, is richly endowed with opiate receptors, and is a particularly good source of this protein since it expresses only the delta type of opiate receptor. In the past year we have isolated many peptides believed to be fragments of opiate receptors and determined their amino acid sequences. We have prepared oligonucleotides corresponding to these sequences and are in the process of screening a cDNA library prepared from NG108-15 cells with these and other probes. We hope to thereby isolate a clone of the opiate receptor cDNA, learn its complete amino acid sequence, elucidate its mechanism of action, and study the regulation of its synthesis and metabolism.

It has become clear that the G protein coupled receptors are members of a closely related family of structures. By the combined use of specific antibodies and synthetic peptides we had earlier characterized a highly conserved receptor domain that activates G-proteins, and showed that no other intracellular domain fully shares these properties. We have in the past year initiated a study of the structural basis of agonist-antagonist discrimination in the related G protein-coupled alpha-2 adrenergic receptor. This protein is coupled to the same G proteins as is the delta opiate receptor and is pharmacologically related as well. We have expressed a cloned alpha-2 adrenergic receptor in both COS and CHO cells and measured receptor numbers by binding assays and receptor coupling by inhibition of adenylyl cyclase and stimulation of GTPase. We found that coupling to G proteins required the presence of an inordinately large number of receptors in the transfected cells, suggesting that a G protein may be limiting. We have therefore prepared COS cells which overexpress each of the two G proteins known to be responsible for opiate receptor and alpha-2 receptor actions. We expect that these cells will allow the more efficient coupling of the two receptors and may also find uses in expression cloning of this type of receptor. Efforts are now also underway to mutate the alpha-2 adrenergic receptor in ways designed to test the role of flexibility and motion in determining the efficacy of agonists.

Project Description and Major Findings:

Opiate receptors, because of their obvious importance to brain function, have been the objects of study of many laboratories, including this one, for a great many years. During this time much has been learned, and yet, almost alone among the receptors this one has not yet been cloned. There seems to be no good reason for this to be so, and there are many reasons for wanting to have the information and research opportunities which a receptor clone will provide. Therefore, over the course of the past year the emphasis of the research program of the entire section has shifted to one in which the major goals are the cloning and expression of opiate and related receptors because it is clear that real progress in understanding this type of receptor will come only after these goals are accomplished.

Protein components of cell surface membranes which receive chemical signals from the cellular environment and transmit this information to the interior are known as receptors. Many of these cell surface proteins, including muscarinic, alpha2-adrenergic, opiate and other peptide receptors share strong structural and functional similarities with one another. These receptors, which interact with a number of hormones and neurotransmitters, are coupled to the modulation of intracellular enzymes by GTP–binding regulatory proteins (G proteins). Through activation of G proteins information is transmitted from neighboring cells and the fluid environment to the internal biochemical machinery. We have concentrated our efforts on the ∂ opiate receptor found in brain and, in a functionally pure form in the neuroblastoma x glioma hybrid cell line, NG108-15. Our goal is to dissect this system into its component parts, and study each of the components of the system in isolation and as reconstituted functional entities.

The neuroblastoma x glioma hybrid cell line, NG108-15, is richly endowed with opiate and other receptors. These are known to activate different G proteins and to ultimately affect different cellular processes. The cells express opiate receptors of only a single type, namely ∂, and do so in large numbers (200,000/cell). They therefore have many advantages over most other tissues, which express at least three different opiate receptor types, and in smaller amounts as well. The ∂ receptors are coupled, primarily via Gi2, as inhibitors, to adenylate cyclase both in these cells and in brain as well. Occupancy 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 response results from an 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. Importantly, it has been shown by others that activation of opiate and alpha2-adrenergic receptors can block calcium channels in NG108-15 cells in a process mediated by Go rather than Gi2.

We had earlier developed procedures for the solubilization of receptors from membranes by extraction with the zwitterionic detergent CHAPS, and shown that it is possible to purify affinity labeled opiate receptors to a homogeneous state. Such preparations, because of the presence of covalently linked opiates are primarily useful for structural studies, although they have also proven to be useful tools for antibody screening experiments as well. The affinity reagent used in this work has been fentanylisothiocyanate (FIT) or a close relative, superFIT. The reagent makes a covalent bond with the ∂ opiate receptor by acylation of an appropriately placed nucleophilic group. In the course of our current work we observed that high concentrations of thiol can remove FIT from the receptor, although without regeneration of binding activity. The chemistry involved here is not completely understood, but experiments

with model compounds have shown that only adducts of FIT with cysteine derivatives behave this way, wheres adducts with tyrosine and histidine or lysine are unaffected by thiols. Thus, it is likely that FIT is acylating a cysteine residue of the receptor. We have prepared several batches of 100-200 picomoles of pure receptor for sequencing purposes. In preparation for the final stage of this work we first prepared trypsin digests, also on the 100-200 pmole scale, of the related, G protein coupled, receptor, rhodopsin, which is easily available in large amounts, and is of known structure. We separated the peptides present in the digests of rhodopsin or of opiate receptors with micro-bore HPLC columns. In order to be able to handle the small amounts of opiate receptor at our disposal we are working with similarly small amounts of rhodopsin and collect the separated peptides directly onto immobilon filter disks each of which can then directly be subjected to automated microsequencing. The trial experiments with rhodopsin showed that correct sequence results were obtained with as little as 2 pmols of peptide introduced into the Applied Biosystems automated microsequencer. We have now obtained the sequences of several peptides isolated from our opiate receptor preparations. Surprisingly, the peptides isolated in highest yield are those resulting from the autodigestion of trypsin, even though the amount of trypsin present is a small fraction of the amount of opiate receptor. Nevertheless, amino acid sequences have been obtained for a number of peptides which are not fragments of trypsin. These structures need to be viewed with caution because of the very small amounts of the peptides so far recovered from our trypsin digests. We hoped that chemical cleavage experiments, might overcome the very low yields so far experienced in our digestions of the opiate receptor with trypsin, but no useful fragments could be isolated from cyanogen bromide digests. It is not clear why the receptor appears to be highly resistant to proteolysis, but it seems possible that the affinity ligand stabilizes the protein by binding within the putative 7 transmembrane helical framework. A different approach, which appears to be useful, has been to employ Streptomyces Griseus protease B (SGPB) a component of pronase which has chymotrypsin like specificity and the remarkable ability to degrade proteins in 6M guanidinium chloride a solvent which completely denatures most proteins. We purified SGPB and found that the enzyme effectively degraded both rhodopsin and purified opiate receptors. The digests have so far resulted in the isolation in reasonable yield of two new peptides from opiate receptors which we had not previously observed in the trypsin digests. None of the 12 or more peptide sequences which we have so far obtained is obviously related to known receptors, but neither can we say they are not so related. We have therefore prepared oligonucleotides corresponding to the back translated sequences of most of these peptides. In collaboration with Dr. Hemin Chin we have used these oligonucletides and others to screen a cDNA library from NG108-15 cells that he has prepared and shown to contain the cDNA for at least some receptors and for the voltage gated calcium channel controlled by Go. The oligonucleotide sequences that we deduce are highly redundant, and so we have also made probes using the polymerase chain reaction to amplify DNA from NG108-15 using the probes as primers. In addition we have prepared probes and primers from known conserved and sometimes not so conserved regions of previously cloned G protein coupled receptors in the hope that screening the library with some combination of such probes will allow us to isolate an opiate receptor clone. We have not so far been successful in these efforts, but believe that the approaches being used will ultimately be successful. The difficulties which we and others have so far encountered suggest that even in cells like NG108-15, opiate receptor mRNA represents a particularly small fraction of the total library.

The very closely related family of G proteins includes at least 4 types of Gi, two types of Go, and two types of transducin. Receptors coupled to these proteins include, among others, opiate, muscarinic and alpha2-adrenergic receptors and the retinal (or light) receptor, rhodopsin. Receptors of this class, which must all interact with very similar regulatory proteins, should share structural features responsible for these interactions. We previously found that one of 46 different anti- rhodopsin monoclonal antibodies tested also recognizes opiate receptors. The epitope against which this antibody is directed corresponds to the fourth cytoplasmic segment of the rhodopsin molecule immediately following the seventh transmembrane helix. This peptide

sequence appears to form a positively charged amphipathic helix which is well conserved in all the G protein coupled receptors studied so far. Peptides corresponding to this structure, stimulate the GTPase activity of a number of purified G proteins, but to somewhat different extents, depending on the protein. These experiments suggest this structure, which is bounded by a conserved and palmitoylated cysteine residue, corresponds to an important part of the receptor domain directly responsible for information transmission and show that this isolated domain can work in a completely defined system. We have studied peptides corresponding to each of the extra- and intra-cellular domains of rhodopsin. Our observations with these materials show that only the domain originally identified in our antibody studies has a marked ability to activate G proteins. We noted that several proline residues, located at specific positions within the putative transmembrane helices 4-7, are conserved among all G protein coupled receptors. Because proline cannot be incorporated within an alpha helix without introducing a bend or kink, these strategically located amino acids must play an important structural role. It seems likely that conformational flexibility, which might be a factor in agonist activity, is introduced by this feature and by the simultaneous occurrence of conserved glycine residues in other helices. We have initiated efforts to exploit opportunities available to test these ideas by mutagenesis experiments with cloned receptors. Because opiate receptors have not yet been cloned, we have made use of a rat alpha2-adrenergic clone that we believe will turn out to be closely related in structure because of extensive functional similarities. We have expressed one such clone transiently in COS cells and found that although the receptor is expressed at the high level of 2 pmols/mg protein, it is not measurably coupled to G proteins. When expressed in CHO cells, we found that the receptor only appears to be coupled to a G protein when there are at least 7 pmols/mg protein. This very high level of expression, although it has been achieved, is not common and suggests that the G proteins present in these cells may not be available for facile coupling. We have therefore prepared COS cell lines which overexpress either Go or Gi-2, proteins known to mediate one or another of the activities of opiate and alpha2-adrenergic receptors. This has been accomplished by transfection with plasmids containing one or the other G protein gene that had originally been prepared in the laboratory of Allen Spiegel. With these cells we expect to be able to study the activities of normal and mutated receptors after transient transfection.

Even though we and others mounted serious efforts to obtain crystals of the alpha subunits of several G proteins in a form suitable for X-ray crystallography, none have so far been produced. Because of the large size of the proteins involved, the information obtainable by this method cannot presently be gained in any other way and is critical to a real understanding of receptor-G protein interactions. In our studies it has become clear that there are major differences between the solubilities of the GDP and GTP liganded states of the proteins. These differences, which may reflect previously observed spectral differences between the two states of G proteins, suggest that the G proteins may be particularly prone to large conformational fluctuations. Such a tendency may also account for the failure of so many laboratories, including our own, to obtain crystals of the proteins even after very extensive efforts. Efforts to stabilize a particular conformation by including peptide fragments of receptors in the crystallization trials have not, so far, been successful. We have therefore initiated spectroscopic studies of the conformational properties of fragments of the G proteins. In related work, we continue to study methods of predicting the secondary structures of proteins from amino acid sequence information. At present, the best such methods have accuracies no greater than 60-65% and so are of limited usefulness. In collaboration with Hayden Coon, we have written several neural net algorithms for making such predictions. The apparent limiting plateau of 60% accuracy may reflect the importance of long range interactions in determining conformation which must therefore be included in predictive schemes.

Significance to Biomedical Research and the Program of the Institute:

Understanding the mechanism of signal-response coupling across cell membranes is a major problem in neurobiology. Cells communicate with one another and with their environment largely by means of chemical messengers which are sensed by cell surface receptors and elicit other chemical changes within the cell. The opioid peptides, norepinephrine, 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 and alpha2-adrenergic receptors and the regulatory proteins with which they interact. In the next year we hope to use sequences of peptide fragments of opiate receptors to clone the receptors. We also plan to prepare a series of mutated forms of alpha2-adrenergic and, ultimately, opiate receptors in which features of the transmembrane helices are changed so that the hinges needed for agonist induced mobility and conformational change are modified. In this way we hope to be able to engineer predictable changes in the pharmacology of these critically important molecules in neuronal function.

Publications:

Ruis, R.A., Streaty, R.A., Loh, Y.P., and Klee, W.A.: Development of G Proteins That Differentially Modulate Adenylyl Cyclase Activity in Mouse Brain. FEBS Letters 288, 51-54 (1991)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-MH-02620-01 LMB

PERIOD COVERED
October 1, 1991 to September 30, 1992

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

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

P.I.:	J.W. Marsh	Research Chemist	LMB, NIMH
Others:	S.K. De	Staff Fellow	LMB, NIMH

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M. V. Eiden, Staff Fellow, LCB, NIMH
J. V. Garcia, St. Jude Child. Res. Hos., Memphis, TN

LAB/BRANCH
Laboratory of Molecular Biology

SECTION
Section on Biophysical Chemistry

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.1	0.7	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.)

The objective of this study is to elucidate the intracellular interactions of the Human Immunodeficiency Virus (HIV) regulatory protein, Nef, with host cell activities. Early in the HIV-1 infective process this retrovirus expresses regulatory proteins, with the Nef transcript representing nearly 80% of total viral mRNA. Recent work by Desrosiers and colleagues has shown that in vivo infectivity by the closely related simian form of HIV is achieved in the absence of Nef expression, but that there is an absolute requirement for Nef in the development of the immunodeficiency. This essential activity of the Nef protein in the development of AIDS has not been defined.

Nef expression in T cell lines has been achieved by transduction with a retroviral expression vector. Nef expression was found to down-modulate the surface expression of CD4 and CD8aß, the two molecules known to interact with the T cell specific tyrosine kinase, $p56^{lck}$. Additionally, we have found that Nef expression in a murine T cell hybridoma sensitizes that cell to T cell receptor stimulation; that is, Nef lowers the biochemical threshold for T cell activation. To elucidate this Nef activity we have developed a murine T cell hybridoma line in which activation, as defined by the T cell specific synthesis of IL2, is dependent upon Nef expression. With this cell line we have found that Nef functions as or through a tyrosine kinase.

HIV NEF FUNCTION

Objectives:

Infection of humans by HIV and of certain primates by SIV (simian immunodeficiency virus) leads to an immunodeficiency (AIDS) with secondary, opportunistic disease and death. Early in the infective process these retroviruses express regulatory proteins, with the Nef transcript representing nearly 80% of total viral mRNA. Recent work by Desrosiers and colleagues has shown that *in vivo* SIV infectivity is achieved in the absence of Nef expression, but that there is an absolute requirement for Nef in the development of the immunodeficiency. This essential activity of the Nef protein in the development of AIDS has not been defined. The objective of this study is to elucidate the intracellular interactions of the Nef protein with host cell activities.

Major Findings:

Nef expression in murine T cell lines has been achieved by transduction with a retroviral expression vector. We chose to use murine T cells since the biochemical pathways for human and murine T cell function are highly homologous, and since the resources for studying murine T cell function far overshadow the possibilities of human studies. Nef expression was found to down-modulate the surface expression of CD4 and CD8aß, the two molecules known to physically interact with the T cell specific tyrosine kinase, $p56^{lck}$. Additionally, we have found that Nef expression in a murine T cell hybridoma sensitizes that cell to T cell receptor stimulation as achieved by the addition of anti-T cell receptor or anti-Thy1 antibodies; that is, Nef lowers the biochemical threshold for T cell activation, where activation is defined by the T cell specific synthesis of IL2. To elucidate this Nef activity we have developed a murine T cell hybridoma line in which activation is dependent upon Nef expression. In the absence of Nef expression this cell line can not generate measurable levels of IL2. This Nef-dependent activation pathway was shown to require a tyrosine kinase activity.

Significance to Biomedical Research:

Nef is non-essential for HIV and SIV infectivity and viral replication, but it is essential in SIV-mediated AIDS. As the simian immunodeficiency virus is markedly homologous to HIV, one should anticipate a similar function for Nef in the development of human disease. Understanding what differentiates HIV from other more benign retroviruses will be essential to the treatment of AIDS.

Proposed Course:

The precise placement of Nef activity in the T cell activation pathway is an ongoing effort. This requires a biochemical dissection of the activation pathway; additionally, we plan to examine different Nef isolates, human and simian, as well as to use site-directed mutagenesis of the Nef gene, to define the structural features responsible for these activities. The examination of Nef structural requirements will utilize the developed Nef-dependent T cell line. Furthermore, as the biochemical activation pathway in T cells is remarkably similar to that of nonlymphoid cells, such as those found in the CNS, and as the infective process of HIV leads to neurological dysfunction in a majority of patients, we plan to examine the effect of Nef on microglial, glial, and neural cell function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01-MH-02621-01

PERIOD COVERED: October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.): Cell Specific Targeting of Biological Agents

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

P.I.:	J. W. Marsh	Research Chemist	LMB, NIMH
	P. T. Moran	Lab Animal Technician	AHCS, NINDS

COOPERATING UNITS (if any)

Laboratory of Cell Biology, NIMH (Dr. M.J. Brownstein)

LAB/BRANCH: Laboratory of Molecular Biology

SECTION: Section on Biophysical Chemistry

INSTITUTE AND LOCATION: NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	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.)

The general aim of this project is to develop antibody-based reagents capable of altering cell-specific functions. This work utilizes cell-specific monoclonal antibodies conjugated to either toxins or recombinant retroviral gene delivery vectors.

One of the approaches in this project is the development of antibody-toxin conjugates capable of eliminating specific immunological in vivo responses. The targeted cells are helper T cells expressing specific Vß chains in their T cell receptor. The Vß chain is responsible for the recognition of various Staphylococcal and Streptococcal enterotoxins, and for the development of shock syndrome arising from this interaction. We have constructed an antibody-toxin conjugate utilizing a monoclonal antibody that recognizes the Vß8.1-8.2 T cell receptor. We have found that this conjugate specifically deletes T cells expressing the Vß8 T cell receptor chain, resulting in a dose-dependent elimination of in vivo $V\beta8^+$ T cell function and of enterotoxin-induced morbidity.

The second aspect of this effort is to determine the potential for antibody-mediated retroviral infection. This laboratory's work with protein toxin conjugates has involved altering the specificity of the normally non-specific plant or bacterial toxins. The specificity has been determined by the chemically coupled antibodies or other ligands. As an extension of this laboratory's expertise in the directed cell delivery of toxins, a project concerned with cell-specific viral infection has been initiated. We will be altering the viral surface glycoprotein (gp70), where binding specificity has been demonstrated, by both chemical and molecular biological means. These chemical modification methodologies will be coincidental with those that the laboratory has developed for toxin moieties. To evaluate the efficiency of the infective process we have established highly sensitive and easily automated assays for transduced reporter gene expression in the targeted cells.

PHS 6040 (Rev. 1/84) GPO 914-918

CELL SPECIFIC TARGETING OF BIOLOGICAL AGENTS

Objectives:

The development of novel antibody-based biological reagents capable of altering cell-specific functions is the general goal for this project. Our approach is to either eliminate specific cellular subpopulations by administration of an antibody-toxin conjugate, or to add new or modify present functions by introduction of a retroviral-based gene vector made cell-specific by conjugation of the retroviral particle with a cell-specific monoclonal antibody.

We have previously demonstrated the potential of antibody-toxin conjugates for the *in vivo* elimination of unwanted cell populations. We have also described the complications in using antibodies recognizing surface antigens that are shared by cell populations for which elimination is not required. That is, the overall *in vivo* efficacy is lowered by increases in the number of targeted cells. As a model system for *in vivo* immunotoxin activity, we have chosen an immunological disorder that is mediated by an easily definable subpopulation of helper T cells. Staphylococcal and Streptococcal enterotoxins can cause an aberrant T cell activation in humans and mice that results in immunological disorders such as toxic shock syndrome, arthritis, or rheumatic fever. This is due to the enterotoxin's interaction with specific Vß T cell receptor chains. Mice possess 20 genes encoding the various Vß chains of the T cell receptor (humans possess a higher level), although individual strains express lower numbers, and individual T cells express only one isotype. Thus, subpopulations of T cells are affected by the various enterotoxins. For example, the Staphylococcus toxic shock syndrome toxin-1 interacts specifically with human Vß2, and Staphylococcus enterotoxin B interacts with murine Vß8. Due to this high specificity we can assess the *in vivo* efficacy of immunotoxins directed at specific Vß chains.

The laboratory's work with protein toxin conjugates has involved altering the specificity of the normally non-specific plant or bacterial toxins. The new specificity has been determined by antibodies or other ligands that are chemically coupled to the toxin. The resultant toxin conjugate has the capacity to specifically kill targeted cells. However, there are clinical conditions where the introduction of new genes into specific cells, or the suppression of gene expression, is more desirable. Murine leukemia viruses have proved to be efficient agents for the transfer of functional genes into a host cell's genome. Like protein toxins, however, these viruses lack cell specificity. This has limited the therapeutic utility of this procedure. As an extension of this laboratory's expertise in the directed cell delivery of toxins, a project whose goal is cell-specific viral infection has been initiated. We will alter the viral surface glycoprotein (gp70), where binding specificity has been demonstrated, by both chemical and molecular biological means. These chemical modification methodologies will be coincidental with those that the laboratory has developed for toxin moieties. As we design retroviral vectors with cell specificity, we hope to learn something about the viral infection mechanisms, especially those features relevant to tropism.

Major Findings:

We have made an Diphtheria toxin conjugate utilizing an anti-Vß8 T cell receptor specific monoclonal antibody. We have shown that this conjugate specifically depletes T cells expressing the Vß8 T cell receptor chain, and remarkably, activated T cells possess increased sensitivity to the immunotoxin activity in spite of decreased surface T cell receptor number. Injection of Staphylococcus enterotoxin B into mice results in activation of $V\beta8^+$ T cells, and leads to elevated TNF levels and a toxic shock syndrome. Preliminary work has demonstrated an immunotoxin dose-dependent elimination of *in vivo* $V\beta8^+$ T cell function and of enterotoxin-induced morbidity.

To determine the capacity for a retrovirus-antibody conjugate to transduce new genes into targeted cells, we have developed quantitative assays that can be efficiently done in large numbers. One is the

micro-adaptation of an enzymatic assay for murine leukemia virus reverse transcriptase, which we now use to enumerate generated viral particles. Another assay was developed to detect (and quantitate) the expression of the reporter gene, beta-galactosidase. This assay is achieved with a 96 well plate reader, and can detect expression by less than 1% of the cellular population. The assay is useful over a six log range of enzyme concentration.

Significance to Biomedical Research:

The abilities to eliminate undesirable *in vivo* cellular functions with an antibody-toxin conjugate, or to incorporate new functions via transduction with an antibody-retrovirus conjugate have obvious clinical applications. However, there are more immediate returns of a more basic nature. The assay developed to detect retroviral transduction of reporter ß-galactosidase genes has already been incorporated into a method for identifying ligands that bind to cloned Gs- or Gi-coupled receptors. The retroviral work should also bring greater definition to the mechanisms of tropism.

The benefits of the antibody-toxin work are two-fold. First, it permits a full description of the *in vivo* capacities of an immunotoxin to change an immunological function. As the immunological function of the immunotoxin-targeted sub-population of T cells is easily assayed by their response to Staphylococcus enterotoxin B, this system permits a quantitative approach to defining immunotoxin *in vivo* activities, capacities, and limitations. Secondly, the various Staphylococcal and Streptococcal toxins interact specifically with various Vß T cell receptor chains in humans and mice, and due to Vß chain homologies between the two species, certain enterotoxins, such as Staphylococcus enterotoxin B, cause shock syndromes in both humans and mice. Thus, the findings from this murine study may have rather direct applications to human disease.

Proposed Course:

The quantitative relationship between antibody-toxin conjugate-mediated T cell depletion and elimination of an immunological response needs to be determined. This *in vivo* work will involve enumeration of viable $V\beta8^+$ T cells by flow cytometry and evaluation of $V\beta8^+$ T cell function by injection of Staphylococcus enterotoxin B. The potential for clinical applications will also be determined.

With the various assays for quantitation of retroviral infection now developed we need to identify and optimize methods for antibody attachment to the virus particles. As with the conjugation of an antibody to protein toxins, there will be a trade-off between the conjugation-mediated loss of viral infectivity and the antibody-mediated increase in cell specificity.

Publications:

Yan, Bai, P.D. Baker, C.H. Evans, and J.W. Marsh: Influence of Endogenous Thy1.1 Cells upon the Efficacy of an Anti-Thy1.1 Antibody-Diphtheria Toxin Conjugate, Bioconjugate Chem. (1991) 2:207.

M. Konig, L. Mahan, J. W. Marsh, J. S. Fink, and M. J. Brownstein: Method for Identifying Ligands That Bind to Cloned Gs- or Gi-Coupled Receptors, Molec. and Cell. Neuro. (1991) 2:331.

Patent Application:

M. Konig, J. W. Marsh, L. Mahan, M. J. Brownstein, J. S. Fink, "A Method of Identifying Ligands and Agonists of Ligands", October 1, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01031-24 LNC

PERIOD COVERED

October 1, 1991 through September 30, 1992

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)*

PI:	Seymour Kaufman	Chief	LNC	NIMH
	Xiang-Jiao Yang	Visiting Fellow	LNC	NIMH
	John Giovanelli	Research Chemist	LNC	NIMH

COOPERATING UNITS *(if any)*

John Donlon University College, Galway, Ireland

LAB/BRANCH

Laboratory of Neurochemistry

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ADAMHA, NIMH Bethesda, MD 20892

TOTAL STAFF 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 unreduced type. Do not exceed the space provided.)*

Phenylalanine administered to rats increases the state of phosphorylation of hepatic phenylalanine hydroxylase with concommitant activation of the enzyme. The synthesis of hepatic phenylalanine hydroxylase is decreased in rats by feeding the animals gluconeogenic metabolites such as glycerol or fructose.

Project Number Z01 MH 01031-24 LNC

Title: The Conversion of Phenylalanine to Tyrosine

Project Description:

The objective of this research project is the detailed description of the enzyme system that is responsible for the conversion of phenylalanine to tyrosine. The specific goal is the analysis of the structure, mechanism of action, and modes of physiological regulation of the essential components in the hydroxylation system. These components include phenylalanine hydroxylase, dihydropteridine reductase, 4a-carbinolamine dehydratase and tetrahydrobiopterin (BH_4).

One of the reasons why the regulation of this system is of special interest to neurochemists is that it can serve as a paradigm for the dynamic interaction between metabolic events in peripheral organs and the brain. When this interaction goes awry, as it does in classical phenylketonuria, it can lead to severe mental retardation.

Major Findings:

During the past year, we have investigated two different aspects of the in vivo regulation of phenylalanine hydroxylase. In one of these studies, we have brought to completion an investigation of the role of phenylalanine in the in vivo regulation of rat liver phenylalanine hydroxylase. We had previously demonstrated that the pure enzyme is activated by cyclic-AMP-mediated phosphorylation and that phenylalanine can stimulate this process. This effect of phenylalanine is distinct from the direct activation of phenylalanine hydroxylase by phenylalanine. We have been investigating the important question of whether this stimulation by phenylalanine of the phosphorylation of phenylalanine hydroxylase occurs in vivo and have demonstrated that it does. Injection of rats with phenylalanine leads to a four-fold increase in phenylalanine hydroxylase activity associated with an eight-fold increase in the level of phosphorylation of the enzyme. These results demonstrate that a substantial fraction of the phenylalanine-mediated activation of hepatic phenylalanine hydroxylase in vivo is secondary to the phenylalanine-dependent increase in the state of phosphorylation of the hydroxylase. This effect constitutes a key part of the redundant control by phenylalanine of phenylalanine hydroxylase activity in the whole organism.

In the second project, we have extended our previous studies of the effect of diabetes on the activity of phenylalanine hydroxylase in rats. We had previously shown that streptozotocin-induced diabetes first activates hepatic phenylalanine hydroxylase by increasing its level of phosphorylation, and subsequently increases the amount of enzyme protein. Others had shown that feeding rats a high protein diet also

increases the activity of phenylalanine hydroxylase. Since both of these conditions favor gluconeogenesis, it was of interest to explore the mirror-image question, i.e., would the provision of alternate convenient gluconeogenic substrates, such as glycerol or fructose have the opposite effect on phenylalanine hydroxylase activity? We have found that feeding rats a diet high in either of these gluconeogenic substrates does, indeed, depress the activity of both hepatic and kidney phenylalanine hydroxylase. The effect of glycerol on phenylalanine hydroxylase activity was shown to be due to a decreased rate of synthesis of phenylalanine hydroxylase. With each of these conditions, the change in the concentration of BH_4 parallels the changes in phenylalanine hydroxylase activity: hepatic BH_4 levels are increased in diabetic rats and markedly decreased in rats fed glycerol or fructose.

Significance to Biomedical Research:

The demonstration that elevated blood levels of phenylalanine increase the activity of phenylalanine hydroxylase by increasing its state of phosphorylation establishes one of the important mechanisms that the organism uses to minimize the postprandial increase in blood phenylalanine concentrations. This is one of the key regulatory processes that protects the brain from damage that would be caused by persistent hyperphenylalaninemia.

The decrease in phenylalanine hydroxylase and BH_4 levels observed in glycerol - or fructose - fed rats further delineates the physiological and nutritional conditions under which phenylalanine hydroxylase-mediated gluconeogenesis contgributes to total body synthesis of glucose. These observations support the hypothesis that glucose derived from the catabolism of phenylalanine makes a significant contribution to the normal nutrition of the brain. Future studies will continue to explore the in vivo regulation of both hepatic and kidney phenylalanine hydroxylase.

Publications:

1. Davis MD, Kaufman S. Studies on the partially uncoupled oxidation of tetrahydropterins by phenylalanine hydroxylase. Neurochem Res 1991; 16:813-9.

2. Citron BA, Davis MD, Kaufman S. Purification and biochemical characterization of recombinant rat liver phenylalanine hydroxylase produced in *Escherichia coli*. Prot Expr Purif 1992; 3:93-100.

3. Davis MD, Kaufman S, Milstien S. Distribution of 4a-hydroxytetrahydropterin dehydratase in rat tissues. FEBS 1992; 302:73-6.

4. Tipper JP, Kaufman S. Phenylalanine-induced phosphorylation and activation of rat hepatic phenylalanine hydroxylase *in vivo*. J Biol Chem 1992; 267:889-96.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: 01 MH 01032-24 LNC

PERIOD COVERED

October 1, 1991 through September 30, 1992

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
	Paula Ribeiro	Visiting Associate	LNC	NIMH
	Yue-hua Wang	Visiting Fellow	LNC	NIMH
	Georg Johnen	Visiting Fellow	LNC	NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	

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.)*

Rat pheochromocytoma tyrosine hydroxylase was cloned, expressed in E.coli, and subsequently purified to homogeneity. The pure recombinant hydroxylase exhibited the same kinetic behavior as that of the activated native enzyme. This activation was reversed, however, when the enzyme was treated with one of the catecholamine products, dopamine. Dopamine was shown to bind with high-affinity to the recombinant enzyme and to cause a significant change in the kinetic as well as spectral properties of the enzyme. The results explain the activation of the recombinant enzyme by the absence of dopamine in E.coli, and the consequent lack of dopamine-mediated down regulation of the hydroxylase. Furthermore, the date identify dopamine binding and inhibition as an essential eukaryotic post-translational modification that serves to regulate tyrosine hydroxylase activity. Several deletion mutants of tyrosine hydroxylase were expressed in E.coli and partially purified for further characterization. The mutant enzyme forms displayed different levels of catalytic activity and very distinct kinetic properties, which were correlated to the size and location of the deletion. The results have helped to redefine the boundaries of the catalytic domain and to clarify the role of the N-terminus in directing substrate specificy, cofactor binding and product inhibition. Other studies have continued to explore the mechanism of tyrosine hydroxylase phosphorylation and dephosphorylation in intact rat striatal synaptosomes. Earlier evidence unveiled a pathway of dephosphorylation for tyrosine hydroxylase which was markedly stimulated by BH_4 in situ. Current studies are attempting to identy the specific phosphoamino acid target sites for BH_4.

Project Number: Z01 MH 01032-24 LNC
Title: Biosynthesis of Catecholamines

Project Description:

The aim of this project is to characterize the hydroxylation reactions that mediate the biosynthesis of catecholamine neurotransmitters, including dopamine and noradrenaline. One line of research has focused on the physical and biochemical properties of tyrosine hydroxylase, the rate-limiting enzyme in this biosynthetic pathway. Other studies have addressed the properties and regulation of tyrosine hydroxylase in intact synaptosomes, with a view to identifying molecular mechanisms that are physiologically significant.

Major Findings:

Rat pheochromocytoma tyrosine hydroxylase was cloned and expressed at high levels at *E.coli*. The recombinant enzyme was purified to apparent homogeneity and subsequently characterized. The kinetic behavior of this hydroxylase was essentially similar to that of the native enzyme, except that the cloned tyrosine hydroxylase appeared to have been purified in an activated form. Subsequent studies on the nature of this activation suggest that the unusual behavior of the cloned enzyme may be explained by the absence of dopamine in *E. coli*, and the consequent lack of dopamine-mediated down regulation of the hydroxylase.

To explore further the role of dopamine in the regulation of tyrosine hydroxylase activity, we studied the binding of dopamine to the recombinant enzyme and the effect of this binding on the kinetic behavior and state of activation of the enzyme. The results demonstrated that high-affinity binding of dopamine to the iron center of the enzyme reversed the kinetic activation and changed the visible spectrum of the enzyme to resemble that of native tyrosine hydroxylase. The data suggest that catecholamine binding and subsequent enzyme inhibition are eukaryotic post-translational modifications that serve to maintain tyrosine hydroxylase in a regulated state of low activity.

In other studies designed to investigate the functional organization of tyrosine hydroxylase, we engineered a series of N-terminus and C-terminus deletion mutants that were cloned, expressed in *E. coli* and partially purified. The relationship between the level of catalytic activity in the mutants and the magnitude of the deletion mutations provided new insight on the regional boundaries of the catalytic and regulatory domains. In addition, by comparing the kinetic properties of the various mutants in relation to the wild-type, we found that the N-terminus imposes a constraint on substrate and cofactor binding but facilitates the binding of dopamine and subsequent enzyme inhibition. This confirms the role of the N-terminus as the

regulatory domain of tyrosine hydroxylase.

In addition to *in vitro* studies, the properties of tyrosine hydroxylase have been investigated in intact rat brain synaptosomes. Previously, we reported that the cofactor tetrahydrobiopterin (BH_4) played a role in the regulation of the state of phosphorylation of tyrosine hydroxylase. In subsequent studies we found that the phosphorylation of tyrosine hydroxylase occurs at multiple sites that are substrates for different protein kinases in the intact synaptosome. Current attempts to identify the specific phosphoserine target for the effect of BH_4 will help to elucidate the molecular mechanism(s) underlying this form of regulation.

Significance to Biomedical Research and Proposed Course of Research:

Our laboratory has dedicated considerable effort to the cloning and purification of the rate-limiting enzyme in the biosynthesis of catecholamine neurotransmitters. One significant consequence of these studies is that sufficient amounts of pure tyrosine hydroxylase, both native and recombinant, have now been made available for extensive characterization of this essential enzyme. This has contributed greatly to our understanding of how catecholamines are synthesized and how their production and output are regulated *in vivo*.

Studies of purified tyrosine hydroxylase have been complemented by studies of dopamine biosynthesis of intact synaptosomes. Synaptosomes are known to retain virtually all the characteristics of intact nerve tissue and thus offer an effective biological preparation for the investigation of the relationships between neural activity and the life cycle of the neurotransmitter. Our ongoing investigation into the regulation of synaptosomal tyrosine hydroxylase has shown that different protein kinases mediate the phosphorylation of specific serine targets *in situ*. Future research will continue to explore the role of phosphorylation of tyrosine hydroxylase in the auto- and heteroregulation of neurotransmitter synthesis and output.

Publications:

1. Ribeiro P, Pigeon D, Kaufman S. The hydroxylation of phenylalanine and tyrosine by tyrosine hydroxylase from cultured pheochromocytoma cells. J Biol Chem 1991; 266:16207-11.

2. Wang Y, Citron B, Ribeiro P, Kaufman S. High-level expression of rat PC12 tyrosine hydroxylase cDNA in *E. coli*: purification and characterization of the cloned enzyme. Proc Natl Acad Sci 1991; 88:8779-8783.

3. Ribeiro P, Wang Y, Citron B, Kaufman S. The regulation of recombinant rat tyrosine hydroxylase by dopamine. Proc Natl Acad Sci 1992 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01038-24 LNC

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	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.)*

Patients with a variant form of phenylketonuria who excrete elevated amounts of 7-biopterin have mild hyperphenylalaninemia. The reason why their metabolism of phenylalanine is impaired is because 7-tetrahydrobiopterin, the presumed precursor of 7-biopterin, is a potent inhibitor of hepatic phenylalanine hydroxylase.

Project Number Z01 MH 01038-24 LNC
Title: Phenylketonuria and Other Diseases Caused by Defects in Biopterin-Dependent Enzymes

Project Description:

The goal of this project is the detailed description, at the molecular level, of physiological effects regulated by components of the aromatic amino acid hydroxylating systems.

Major Findings:

During the last year, we have continued our studies of aspects of the phenylalanine hydroxylase system that might be relevant to understanding the cause of hyperphenylalaninemia (HPA) in a new variant form of this disease. As described in last year's report, this new form of HPA appears to be caused by a deficiency of phenylalanine hydroxylase stimulator protein (PHS), an enzyme discovered in the Laboratory of Chemistry almost 20 years ago, and is characterized by the excretion of markedly elevated amounts of 7-biopterin. In vitro experiments showed that in the absence of PHS, there is a phenylalanine hydroxylase-stimulated conversion of the naturally-occurring hydroxylation cofactor, tetrahydrobiopterin (BH_4) to an isomer, 7-BH_4. The basis of the HPA in PHS-deficient patients, however, has remained mysterious.

We have now shown that 7-BH_4 at concentrations as low as 1μM is a potent inhibitor of hepatic phenylalanine hydroxylase. The inhibition can be overcome either by increasing the concentration of BH_4 or by decreasing the concentration of phenylalanine. These in vitro results, therefore, provide a possible mechanism for the HPA that characterizes patients who excrete 7-biopterin and suggest that even mild restriction of dietary intake of phenylalanine would significantly decrease blood levels of phenylalanine.

Significance to Biomedical Research and Proposed Course of Project:

Confirmation of a deficiency of the phenylalanine hydroxylase stimulator protein in hyperphenylalaninemic children who excrete 7-biopterin should provide information as to its role in aromatic amino acid metabolism. Tissue localization studies as well as cDNA isolation should aid in determining whether this enzyme also plays a similar role in the CNS.

Publications:

1. Bolla KI, Milstien S, Briefel G, Wieler L, Kaufman S. Dihydropteridine reductase activity: lack of association with serum aluminmum levels and cognitive functioning in patients with end renal disease. J Neurol 1991; 41:1806-9.

2. Kaufman S. Some metabolic relationships between biopterin and folate: implications for the "methyl trap hypothesis". Neurochem Res 1991; 16:1031-6.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

01 MH 01039-24 LNC

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

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INSTITUTE AND LOCATION

ADAMHA, NIMH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	

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 cyclohydrolase has been purified to near homogeneity from rat liver and partially characterized. The enzyme appears to be heterogeneous and may contain two different subunits.

Project Number
Z01 MH 01039-24 LNC

Title of Project
Pteridine Biosynthesis

Project Description:
The de novo biosynthetic pathway for tetrahydrobiopterin (BH_4), the cofactor required in the rate-limiting step of biogenic amine neurotransmitter synthesis and phenylalanine metabolism, has been elucidated. The complete characterization of this pathway will aid in the understanding of the role of BH_4 in neuropsychiatric disorders where BH_4 levels have been shown to be decreased, in viral infections where immune stimulation results in increased BH_4 levels, as well as in diagnosis and treatment of genetic defects in BH_4 biosynthesis.

Major Findings:
A new procedure has been developed to purify relatively large amounts of GTP cyclohydrolase. The enzyme from rat liver has a native molecular weight of approximately 200 kD and appears to be composed of both 30 kD and 55 kD subunits.

Significance to Biomedical Research and Proposed Course of Project:
The regulation of tetrahydrobiopterin biosynthesis and in turn the regulation of those systems which are dependent on tetrahydrobiopterin has not yet been well characterized. Understanding of the regulation of these pathways in pathological conditions where tetrahydrobiopterin levels are changed may provide clues for new therapeutic treatments.

Publication:
none

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01040-24 LNC

PERIOD COVERED

October 1, 1991 through September 30, 1992

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
	Bruce Citron	Geneticist	LNC	NIMH
	Jennifer Tipper	Senior Staff Fellow	LNC	NIMH
	Takaaki Kobayashi	Visiting Fellow	LNC	NIMH
	Cynthia Falke	Biologist	LNC	NIMH

COOPERATING UNITS *(if any)*

Robert Pohlman Biology Department, Colgate University, Hamilton, NY
Charles Schwartz Greenwood Genetic Center, Greenwood, SC

LAB/BRANCH

Laboratory of Neurochemistry

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ADAMHA, NIMH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.2	2.2	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.)*

We are studying the aromatic amino acid hydroxylases and nitric oxide synthase (which require tetrahydrobiopterin) and the enzymes responsible for the synthesis of tetrahydrobiopterin through an integrated interdisciplinary approach. This multi-level research is uncovering protein, substrate, effector, and product interactions that are significant in normal and diseased state biology. A central element to our research is the directed mutagenesis of the cloned enzymes to elucidate the structures and specific roles of a variety of catalytic and regulatory sites.

Project Number: Z01 MH 01040- 24LNC

Title of Project: Molecular Biology of the Pterin-Dependent Hydroxylases and Ancillary Enzymes

Project Description:

We are studying the precise molecular interactions important in regulation and catalysis in several pathways: 1. The hydroxylation of aromatic amino acids, 2. The biosynthesis of nitric oxide, and 3. The biosynthesis of the cofactor, tetrahydrobiopterin.

Major Findings:

Phenylalanine Hydroxylase:

We have created thirty mutant rat phenylalanine hydroxylase clones. These include broad varying deletions from the N-terminal and C-terminal ends, scanning insertional mutations at random positions along the length of the protein, and specific alterations of side chains predicted to be involved in the normal function of the hydroxylase. Examination of the kinetic parameters of the altered enzymes has supported the locations of the regulatory and catalytic domains, and indicated that the negative charge, introduced by phosphorylation of the enzyme, is an important factor in activation of the enzymatic activity. The coding sequence of a human phenylalanine hydroxylase cDNA has been subcloned into and expressed in a prokaryotic expression system.

Tyrosine Hydroxylase:

Expression of the recombinant wild-type rat enzyme is enabling the elucidation of regulatory components involved in vivo. Possible models for product inhibition have been discovered by comparing the unmodified recombinant enzyme to the naturally isolated enzyme. Additionally, we have synthesized seven deletion mutants of the enzyme with residues removed from the N-terminus, C-terminus, or both in combination. These mutants have helped define the boundaries of catalytic, regulatory, and interactive domains.

Tryptophan Hydroxylase:

This enzyme is necessary for the synthesis of serotonin and is currently difficult to obtain in sufficient quantities from pineal glands or brain stem. Expressing clones of rabbit and human tryptophan hydroxylase have been assembled a nd confirmed which will lead to the ability to broaden the kinetic analysis of this enzyme and to examine the functionally important motifs in the enzyme.

GTP Cyclohydrolase, 6-Pyruvoyl Tetrahydropterin Synthetase, and Sepiapterin Reductase:

These enzymes are responsible for the biosynthesis of tetrahydrobiopterin, the cofactor in several characterized catalytic steps. We are analyzing purified GTP cyclohydrolase protein to devise a probe to the protein purified in our laboratory, which seems distinct from that reported in the literature. We are quantitating the mRNA levels of each of these enzymes to study their response to such factors as interferon γ.

4a-Carbinolamine Dehydratase:

The first step in the regeneration of tetrahydrobiopterin is the conversion of 4a-carbinolamine tetrahydrobiopterin to quinonoid dihydropterin by this enzyme. Mild hyperphenylalaninemia may occur in individuals lacking this enzyme. We have reverse-transcribed and polymerase-chain amplified coding sequences for this gene from rat liver mRNA.

Project Number: Z01 MH 01040-24 LNC

Nitric Oxide Synthase:

Nitric oxide has recently been implicated as an important messenger in neuron function. We have amplified the full length rat coding sequence for the enzyme and are testing putative clones for the generation of a prokaryotic expression system.

Significance to Biomedical Research and Proposed Course:

Tetrahydrobiopterin is present in almost all mammalian tissues that have been examined. Aromatic amino acid hydroxylases, expressed in a small subset of peripheral and brain cell types, have an absolute dependence on tetrahydrobiopterin. These hydroxylases catalyze rate-limiting steps in the metabolism of phenylalanine and in the biosynthesis of dopaminergic and serotinergic neurotransmitters. Nitric oxide synthase has been implicated in retrograde messenger synthesis in the nervous system and also exhibits a dependency on tetrahydrobiopterin. Tetrahydrobiopterin is also synthesized in many other tissues for functions that remain a mystery but the activity of the biosynthetic pathway seems modulated by viral infections including AIDS.

Deficiencies in phenylalanine hydroxylase can lead to mental retardation. Defects in tetrahydrobiopterin synthesis or maintenance can produce severe central nervous system disorders in addition to mental retardation. Improved understanding of the normal and abnormal function of the pathways which involve tetrahydrobiopterin will be a necessity for full characterization of the molecular interactions in the central nervous system in health and disease.

Examination and comparison of the sequences of these genes, expression of the gene products in unmodified form, nucleic acid quantitation, and directed mutagenesis will greatly facilitate the analysis of the relationships between these enzymes, their substrates, products, and effector molecules. The information accumulated will help identify the specific biochemical alterations in various mental illnesses and aid the development of therapeutic approaches.

Publications:

Citron BA, Davis MD, Kaufman S. Purification and Biochemical characterization of recombinant rat liver phenylalanine hydroxylase produced in Escherichia coli. Prot Expr Purif 1992; 3:93-100.

Wang Y, Citron BA, Ribeiro P, Kaufman S. High-level expression of rat PC12 tyrosine hydroxylase cDNA in Escherichia coli: Purification and characterization of the cloned enzyme. Proc Natl Acad Sci USA 1991; 88:8779-8783.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02563-02 LNC

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Role of Tetrahydrobiopterin in Nitric Oxide Synthesis

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

PI: John Giovanelli Research Chemist LNC NIMH
Seymour Kaufman Chief LNC NIMH
Kenneth Campos Senior Clinical Investigator LNC NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

ADAMHA, NIMH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	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.)*

Various workers have proposed an inverse relationship between the amount of tetrahydropterin (BH_4) bound to nitric oxide synthase and the extent to which activity of the enzyme is stimulated by added BH_4. Critical assessment of the validity of this proposal requires nitric oxide synthase that is free of BH_4. Treatment of the enzyme with acid ammonium sulfate or charcoal (procedures commonly used to resolve enzyme-cofactor complexes) did not remove BH_4 from the enzyme. Nitroblue tetrazolium, an oxidant of BH_4 in solution, did not oxidize enzyme-bound BH_4. These observations suggest that BH_4 is bound in a hydrophobic pocket that is sequestered from the aqueous environment. They further indicate contrary to the proposal of other workers, that enzyme-bound BH_4 is not an intermediary electron carrier in the "diaphorase" activity of nitric oxide synthase, i.e., in the transfer of electrons from NADPH to a dye such as nitroblue tetrazolium. Surprisingly, we found that addition to the assay of the reduced form of the dye, dichlorophenol indophenol, results in a marked increase in the stimulation (up to 40-fold) by added BH_4. The chemical nature of enzyme-bound biopterin under these conditions is now being examined. The most promising approach to clarifying the role of BH_4 in nitric oxide synthase has been provided by studies with substrate amounts of enzyme. Under these conditions, in the absence of NADPH, arginine is converted to a compound tentatively characterized as hydroxyarginine; no citrulline is formed. Synthesis of hydroxyarginine is dependent on Ca^{2+} and calmodulin, and stimulated by BH_4. Thus, NADPH appears not to be the immediate electron donor in hydroxyarginine synthesis, but is required for further metabolism of this intermediate to the end products nitric oxide and citrulline. Experiments are underway to determine the immediate electron donor required for conversion of arginine to hydroxyarginine.

Project Number Z01 MH 02563-02 LNC
Title: Role of tetrahydrobiopterin in nitric oxide synthesis

Project Description:

Nitric oxide synthase (NOS) catalyzes the oxygenation of arginine in the presence of NADPH to form nitric oxide, citrulline and NADP. The enzyme is of great interest because nitric oxide appears to participate in a variety of physiological processes, such as vasodilation, regulation of macrophage anti-tumor and anti-microbial activity, platelet adhesion and cerebellar signaling. Nitric oxide synthesis is enhanced by extremely low concentrations of the natural isomer of tetrahydrobiopterin. The aim of this project is to elucidate the role and physiological significance of tetrahydrobiopterin in nitric oxide synthesis.

Major Findings:

Purified nitric oxide synthase, a homodimer, was found to contain variable amounts of biopterin (0.2 to 0.5 mol/mol subunit), present predominantly as tetrahydrobiopterin (BH_4). Other workers have proposed an inverse correlation between the amount of enzyme-bound BH_4 and the extent of stimulation by added BH_4. Our limited studies indicate only modest changes in the extent of stimulation by BH_4 (approx. 30%) between the extremes in the BH_4 content of the enzyme. Clearly, critical assessment of the validity of this proposal requires nitric oxide synthase that is free of BH_4. Treatment of the enzyme with acid ammonium sulfate or charcoal (procedures commonly used to resolve enzyme-cofactor complexes) did not remove BH_4 from the enzyme. Nitroblue tetrazolium, an oxidant of BH_4 in solution, did not oxidize enzyme-bound BH_4. These observations suggest that BH_4 is bound in a hydrophobic pocket that is sequestered from the aqueous environment. They further indicate, contrary to the proposal of other workers, that enzyme-bound BH_4 is not an intermediary electron carrier in the "diaphorase" activity of nitric oxide synthase, i.e., in the transfer of electrons from NADPH to nitroblue tetrazolium. Surprisingly, we found that addition to the assay of the *reduced* form of the dye, dichlorophenol indophenol, results in a marked increase in the stimulation (up to 40-fold) by added BH_4. The chemical nature of enzyme-bound biopterin under these conditions is currently being examined.

The most promising approach to clarifying the role of BH_4 in nitric oxide synthase has been provided by studies with substrate amounts of this enzyme. NADPH is the ultimate electron donor in the oxygenation of arginine to citrulline and nitric oxide. The overall reaction is known to proceed via two partial reactions: arginine is first converted to the intermediate hydroxyarginine, which is further metabolized to citrulline and nitric oxide. We have found that substrate amounts of nitric oxide synthase in the absence of added NADPH convert arginine to a compound

tentatively characterized as hydroxyarginine. No citrulline is formed. This finding shows that reductant(s) bound to the enzyme can substitute for NADPH in the first, but not the second, step of the overall reaction. Synthesis of hydroxyarginine has been shown to be dependent on Ca^{2+} and calmodulin, and stimulated by BH_4. This is the first experimental demonstration of the cofactors required for this partial reaction. These studies already provide important clues as to the electron donors in the two partial reactions of nitric oxide synthesis. Thus, NADPH appears not to be the immediate electron donor in hydroxyarginine synthesis, but is required for further metabolism of hydroxyarginine to the end products. Experiments are under way to determine whether enzyme-bound BH_4 is the reductant for hydroxyarginine synthesis.

Significance to Biomedical Research and Proposed Course of Research:

One of the most interesting physiological roles proposed for nitric oxide is that it acts as a neurotransmitter in the brain, possibly via stimulation of guanylate cyclase. Studies by other workers indicate physiological links between excitatory amino acid receptor stimulation (by glutamate, NMDA, etc.), nitric oxide formation, and increase in cGMP. Tetrahydrobiopterin may therefore be involved in the physiology of the excitatory amino acid neural pathways in the brain, such as cerebellar signaling and descending frontal cortical pathways. The latter are involved in abulia, amotivational and behavioral disinhibition syndromes, and possibly schizophrenia.

Experiments with substrate amounts of enzyme promise to provide key information on the roles not only of BH_4 but other components of this complicated enzyme. Studies initiated with the first partial reaction (conversion of arginine to hydroxyarginine) will be confirmed, and extended to cover the second partial reaction catalyzing formation of final products. It is expected that an E.coli clone expressing cerebellar nitric oxide synthase will soon become available through collaboration with Dr. Bruce Citron. E.coli does not synthesize BH_4. Therefore, any expressed nitric oxide synthase would be free of BH_4, and allow critical examination of the role of this pterin. We further plan to examine the physiological significance of the effect of tetrahydrobiopterin on nitric oxide synthase in mutant mice defective in synthesis of this pterin.

Publications:

1.Giovanelli J, Campos KL, Kaufman S. Tetrahydrobiopterin, a cofactor for rat cerebellar nitric oxide synthase, does not function as a reactant in the oxygenation of arginine. Proc Natl Acad Sci 1991; 88:7091-5.

2. Campos KL, Giovanelli J, Kaufman S. Studies on the role of tetrahydrobiopterin in rat cerebellar nitric oxide synthase. In: Moncada S, Marletta MA, Hibbs JB Jr., Higgs EA, eds. Biology of Nitric Oxide. Colchester, England: Portland Press (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02564-02 LNC

PERIOD COVERED

October 1, 1991 through September 30, 1992

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

Tetrahydrobiopterin Biosynthesis and HIV Pathogenesis

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

PI:	Sheldon Milstien	Research Chemist	LNC	NIMH
	Naoki Sakai	Visiting Fellow	LNC	NIMH
	Seymour Kaufman	Chief	LNC	NIMH

COOPERATING UNITS *(if any)*

Melvyn Heyes	LCS	NIMH
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LAB/BRANCH

Laboratory of Neurochemistry

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INSTITUTE AND LOCATION

ADAMHA, NIMH Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	1.6	

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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.)*

Previously we showed that there was a strong correlation between increased tryptophan metabolism and increased pterin synthesis in macaques infected with an immunosuppressive D-retrovirus. Investigation of these two biochemical pathways in tissue culture model systems has now enabled us to establish that these pathways are induced in parallel by cytokines and that the metabolism of tryptophan does not require reduced pterin cofactors.

Project Number: Z01 MH 02564-02 LNC

Title of Project
Tetrahydrobiopterin Biosynthesis and HIV Pathogenesis

Project Description:
Dihydroneopterin excretion has been shown to be correlated with activation of the immune system. The increased production of dihydroneopterin probably represents an increased intracellular synthesis of tetrahydrobiopterin which may play a yet to be characterized role in the functioning of the immune system. Recently, we have confirmed that dihydroneopterin can be used as a diagnostic marker for progression of AIDS and possibly for monitoring therapies.

Several model systems are being investigated to study the relationship between the activation of the immune system, as seen in HIV infection, and increased unconjugated pterin synthesis. These include macaques infected with D-retroviruses and poliovirus, rodents treated with immune system activators, and cells in tissue culture.

Major Findings:
Tryptophan metabolism and pterin synthesis has been studied in cytokine-treated macrophages and fibroblasts, have model systems for immune activations in vivo. The rate of increase and dose-dependence for interferon-γ/tumor necrosis factor-α dependent changes in kynurenine and neopterin or biopterin levels were essentially identical. However, experiments with both pterin synthesis inhibitors as well as with cells from patients with genetic lesions in the pterin biosynthetic pathway, unequivocally show that tryptophan metabolism via the kynurenine pathway does not require the presence of any pterin cofactors.

Significance to Biomedical Research and Proposed Course of Project:
The physiological function of increased levels of dihydroneopterin and tetrahydrobiopterin following HIV infection is presently obscure. The only established function of tetrahydrobiopterin is as a cofactor for the hyrdroxylation of the aromatic amino acids. It is possible that abnormal brain levels of pterins could result in neurotransmitter imbalances and play some role in the CNS pathology of AIDS. Elucidation of the function could lead to new pharmacological approaches to treatment involving inhibitors of pterin synthesis.

Publication:
1. Heyes MP, Lackner A, Kaufman S, Milstien S. Cerebrospinal fluid and serum neopterin and biopterin in D-retrovirus infected rhesus macaques (Macaca Mulatta): relationship to clinical and viral status, AIDS 1991;5:555-560.

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

PROJECT NUMBER:
Z01 MH 00851-28 LNP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Brain Mechanisms of Species-Typical Behavior 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 LNP, NIMH

COOPERATING UNITS *(if any)*

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Laboratory of Neurophysiology

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INSTITUTE AND LOCATION
NIMH, ADAMHA, NIH Animal Center, Poolesville, Maryland 20837

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

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

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

The first part of this report describes the purpose and preparation of a monograph on the role of tegmental structures in orchestrating the somatic and autonomic manifestations of species-typical displays of squirrel monkeys (Saimiri sciureus). The monograph will include illustrations of the performance curves and reconstructions of the tegmental lesions in 37 subjects. A short second part makes note of the completion of four invited articles including, respectively, one dealing with the cerebral evolution of emotion for The Handbook of Emotions; a 5000-word article entitled LIMBIC SYSTEM for The Blackwell Dictionary of Neuropsychology; an introductory chapter for a volume The Neurobiology of the Cingulate Cortex and Limbic Thalamus; and an initial article on "The Limbic System Concept" for a volume The Temporal Lobes and Limbic System.

Project Description

Part I. Preparation of monograph on role of tegmental structures in species-typical displays. The experimental work under consideration was originally undertaken to clarify the role of respective medial pallidal projections in the somatic and autonomic manifestations of species-typical displays of squirrel monkeys. Although the main findings included in the present report have been described in previous publications, the large amount of material made it impossible to present more than illustrative examples of key cases and control observations. For the reasons to be explained, it is unlikely that experiments of this kind will be done again. Consequently, it has been concluded that it would be worth while to publish a monograph showing the performance curves and reconstructions of the brainstem lesions on each of 37 animals.

Objectives: For the phase of the study under consideration, it was necessary to place lesions in the parts of the thalamic tegmentum and midbrain tegmentum that contain fiber pathways involved in sustaining functions on which an animal's life depends. In caring for such animals it is as important to know what not to do as it is to be expert in administering treatment. Two considerations are foremost for believing that it is unlikely that circumstances may ever again be so favorable for obtaining the invaluable information derived from the difficult kind of experiments under consideration. In the first place the present rules and regulations would make it impossible to house animals across the hall from the office of the principal investigator for keeping a close eye on the precarious condition of operated animals. This had formerly been the situation at Poolesville, as well as at Bldg. 36 and at the Clinical Center. To control the multiplying spread of organisms and to reduce odors to a minimum, it was mandatory to forbid hosing for cleaning the floors and walls and to use other simple measures. But the most critical factor was the capability of the principal investigator to keep a close eye on such animals over week ends. Nearly half the animals had to be transported elsewhere for this purpose, snowstorms being one of the contingencies. Parenthetically, it should be noted here that despite the current emphasis on finding ways to promote the psychological well being of primates, little attention has been given to what we have observed to be the most important necessity of all. As we pointed out upon publishing a brain atlas of the squirrel monkey (1962, p. 89), "The greatest asset in the maintenance of animals is an animal caretaker who is solicitous of their needs and who by his voice, bodily movement, and methods of handling helps dispel fear."

The other major reason for recording the present material in greater detail is the uncertainty of availability of primates for future research. Because of their size and behavior, squirrel monkeys are especially valuable for neurobehavioral studies on species-typical behavior. But like other infrahuman primates they are threatened

by extinction. For illustration, just consider that when the film of a skull X-ray of one of our squirrel monkeys was placed over a photo of the fossil skull of a like creature estimated as living 25 million years ago, there was almost perfect conformity, right down to the buck-toothed incisors. With the present clearing of tropical forests, what is the likelihood that the ecology will long support such evolutionary stability?

Methods and Major Findings. Terrestrial vertebrates use four main kinds of displays in social communication. In lizards they are identified as signature, aggressive, courtship, and submissive displays. The gothic-type squirrel monkey performs a mirror display that incorporates features of their aggressive, courtship, and greeting displays. Upon exposure to a mirror, the three main features of the display—vocalization, thigh spreading, and full genital tumescence— can be expected at a criterion of 80% or more in sets of 30 trials. After a criterion level of performance, electrocoagulations of selected size and shape are placed in tegmental or neighboring structures under conditions simulating those of a patient operated under anesthesia. Testing resumes upon recovery .

After surgery the 37 animals under consideration were tested twice a day for periods ranging from 4 weeks to 14 months, with a mean of 5.6 months. The illustrations of the performance curves of all 37 monkeys have been prepared for publication. Likewise drawings have been made from the serial sections of all animals showing reconstructions of the lesions at three representative levels. The checking of the drawings against projected brain sections and the labeling is nearing completion. The main findings may be summarized as follows: (1) bilateral interruption involving any of the six so-called transverse pathways of the diencephalon do not interfere with the performance of the display; (2) projections of the medial pallidal segment to the tegmentum are more effective than those to the thalamus in eliminating the display; (3) large supranigral tegmental lesions are the most effective in obtaining and enduring elimination of the display; (4) no particular tegmental structure has been identified with the thigh-spread component; (5) the vocal component is permanently eliminated by the lesions of the periventricular gray involving the origin of the medial longitudinal fasciculus, and there is a marked reduction with lesions involving the periaqueductal gray and

contiguous central tegmental tract; (6) selective interference with the genital component appears to depend on lesions involving the medial forebrain bundle; (7) the entire red nucleus can be eliminated without altering the incidence of full display; and (8) large lesions of serotonergic systems may be produced without affecting the display.

Part II. Preparation of invited articles on cerebral evolution of various functions. Because of the Poolesville contributions in the general area of cerebral evolution and functions of major systems of the forebrain, the PI was asked to contribute four articles listed below under publications. In view of the time consuming nature of this undertaking and the use of laboratory facilities, a brief report should be given of the nature and scope of these articles. As background for these comments it should be noted that the evolutionary transition from reptiles to mammals is characterized by the development of three forms of behavior identified as: (1) nursing conjoined with maternal care; (2) audiovocal communication for maintaining maternal-offspring contact; and playful behavior. These forms of behavior appear to have depended on an expansion of the evolutionarily old cortex enveloping a large cerebral convolution that Broca called the great limbic lobe because it surrounds the brainstem. This lobe and its primary connections with the brainstem constitutes the so-called limbic system (MacLean, 1952). Comparative anatomical and chemical findings indicate that the limbic system represents an inheritance from early mammals. In 1955 Stamm showed that the limbic cortex of the cingulate gyrus is essential for normal maternal behavior. In addition our work at Poolesville has shown that the cingulate cortex is implicated in the production of the separation cry and in playful behavior. Hence one might say that the history of the evolution of the limbic system is the history of the evolution of mammals and their family way of life.

For the foregoing and other reasons the PI was asked to write an introductory chapter for a book entitled "Neurobiology of the Cingulate Cortex and Limbic Thalamus". Another paper in press is a 5000-word article entitled "Limbic System" for The Blackwell Dictionary of Neuropsychology. A third article "The Limbic System Concept" is the first chapter of a book entitled The Temporal Lobes and Limbic System. Finally, a fourth article "Cerebral Evolution of Emotion" is to appear in The Handbook of Emotions.

Significance to Biomedical Research and the Program of the Institute: It has been the traditional neurological view that the basal ganglia of the forebrain (i.e., striatal complex , showing an ancestral origin in reptiles) subserve purely motor functions under the control of the neocortex. A primary purpose of the Poolesville facility opening in 1971 was to conduct

experiments suggested by negative evidence that the accepted view was wholly correct. Experiments on animals as diverse as lizards and monkeys have revealed that the striatal complex is implicated in the reciprocal performance of displays used in social communication. Observations in other studies also are indicative that the striatal complex plays a basic role in orchestrating the daily master routine and subroutines. Although other names are used for the symptomatology, a diversity of clinical evidence is accumulating indicative that the striatum is involved in directing the performance of both the master routine and subroutines.

In regard to the NIMH program, the laboratory findings on the limbic system are most clearly relevant to family related behavior, and particularly separation, a condition that makes being a mammal so painful. In previous reports the relevance has been pointed out to an understanding of the neural substrate of crying and laughter and to such mental health problems as childhood separation anxiety, grief reactions, depression, and various forms of addiction, particularly with respect to the high concentration of opioid receptors in the cingulate cortex. In an evolutionary sense, the roots of the painful aspects of separation are perhaps traceable to the drastic consequences of separation of mother and offspring when nursing became a mammalian way of life. In this connection it is relevant that in a previous project, the PI observed that the cingulate cortex implicated in the separation cry is innervated by nuclei including those involved in the perception of pain. The clinical evidence that the limbic system generally is involved in the experience and expression of emotion is perhaps compatible with the suggestion that the subjective aspects may have been evolutionarily honed by feelings of separation.

Publications:

Book Chapters

MacLean PD. Obtaining knowledge of the subjective brain ("epistemics"). In: Harrington A, ed. So Human a Brain: Knowledge and Values in the Neurosciences. Boston: Birkhäuser Boston Inc, 1992;57-70.

MacLean PD. The limbic system concept. In: Trimble, MR, Bolwig TG, eds. The Temporal Lobes and Limbic System. Petersfield, England: Wrightson Biomedical Publishing, 1992;1-14.

MacLean PD. Cerebral evolution of emotion. In: Lewis ML, Haviland JM, eds. New York: The Guilford Press, in press.

MacLean PD. Evolutionary psychiatry and extrapolative "memory of the future." In: Sorel E, ed Proceedings of XII World Congress of Social Psychiatry, in press.

MacLean PD. Introduction: Perspectives on cingulate cortex of the limbic system. In: Vogt BA, Gabriel M, eds. The Neurobiology of the Cingulate Cortex and Limbic Thalamus. Boston: Birkäuser, in press.

MacLean PD. Limbic System. In: Beaumont JG, Sergent J eds.The Blackwell Dictionary of Neuropsychology. Oxford: Basil Blackwell, Ltd., in press.

MacLean PD. Book review : Darwin on Trial. by Phillip E. Johnson. Washington: Regnery Gateway, 1991, 195 pp. In: Zygon: Journal of Religion and Science, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 MH 01092-14 LNP

PERIOD COVERED

October 1, 1991 to September 30, 1992

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	Chief	LNP, NIMH
Others:			
	Andrew R. Mitz	Biomedical Engineer	LNP, NIMH
	Kazumi Kawahira	Guest Researcher	LNP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Animal Center, Poolesville, Maryland 20837

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.8	2.8	

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

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

We have used the methods of behavioral neurophysiology to study two major divisions of the frontal lobe of primates: the premotor cortex (PM) and the prefrontal cortex (PF). We have just published the first phase of a study on motor learning by showing that PM cells dramatically change their activity while monkeys learn what motor act to execute on the basis of an arbitrary sensory stimulus. The ability to switch the behavioral significance of sensory stimuli in the short term underlies the flexibility that characterizes the adaptation of advanced mammals to a rapidly changing but partially predictable environment. In addition, there has long been a need for more powerful behavioral methods with which to distinguish neuronal activity related to sensory and perceptual processes from those involved in the selection and control of behavior. To this end we have developed two novel behavioral paradigms that distinguish sensory from motor activity in PM and PF neurons. Our results support the hypothesis that PM plays a role in retrieving from motor memory the movement that needs to be made on the basis of a sensory event, but that PF is more involved in spatial analysis and other aspects of sensory information processing.

OBJECTIVES:
We have been studying neuronal activity in two major subdivisions of the primate frontal lobe, the premotor (PM) and prefrontal cortex (PF) to better understand the functional organization of the primate frontal lobe. Specifically, we would like to know how, in mammalian species that have a large number of functionally related yet functionally distinct cortical fields, these areas act together and separately in the selection and control of behavior. In the future, we plan to expand our methodological approach to incorporate the study of behaviors in a more ethologically relevant context. To this end, an objective of this project is to develop methods for the telemetric recording of neuronal activity in relation to the selection and control of communicative motor behavior, especially vocalization.

METHODS:
Behavioral Neurophysiology
Behavioral neurophysiology involves the study of single-neuron activity in awake, behaving animals, in this project, rhesus monkeys. We focus on the study of the nonprimary motor cortex, which consists of the premotor cortex (PM), the supplementary motor area (M2) and the prefrontal cortex (PF). The methods of the first 9 subprojects and the results of our electroanatomical and architectonic studies, which have helped define the boundaries and internal organizations of the functionally distinct parts of the PM and M2, have been detailed in past annual reports. Accordingly, this year's report details the methodological aspects of subprojects 10-14, which are those subprojects upon which publications have appeared this year or work has been ongoing.

Subproject #10. The properties of PM and PF neurons were contrasted. The monkey began each behavioral trial by contacting the central of three touch pads. Next, a 1 sec-long red or green stimulus appeared directly in front of the monkey, followed by a 1.25 sec delay. Then one red and one green light simultaneously appeared peripherally, each above a potential target. The monkey withheld its response for an *instructed delay period* of 1.25 sec, then touched the pad under the peripheral cue matching the first stimulus. The purpose of this experiment was to compare PF and PM activity in a single individual. Previous comparisons of PF and PM activity have been methodologically flawed, being based on comparison among different species of monkey, different individuals, different behavioral tasks, and using different recording techniques.

Subproject #11. We have trained four monkeys to move a lever to one of three positions in response to complex, color stimuli. The set of stimuli was changed periodically to restart the learning process. We studied changes in neuronal activity as the animal learned which stimulus instructed each possible response. The prevailing hypothesis concerning PM function holds that one of its roles is to recall, from a memory store, an appropriate motor program based upon recognition of a stimulus or, more generally, environmental context. That hypothesis predicts that the response of a PM unit would change as a new stimulus-response association was established, and that the final pattern of activity, once that new association was learned, would resemble the activity of the same cell during equivalent responses to familiar figures.

Subproject #12. We operantly conditioned a rhesus monkey to grasp a handle beneath a transparent plastic surface containing a circular pattern of eight light emitting diodes (LEDs). There was an additional LED in the center of the circle. Following an intertrial interval, the center LED was illuminated and the animal had to look directly at the LED and "capture" it with the handle beneath the LED for 1 sec. Next, one of the eight LEDs was illuminated for 500 msec, an event termed the *prime stimulus* presentation. Then a series of 0 to 4 *distractor* stimuli (each 100 msec in duration) were successively presented in different positions separated by delays of 550 or 750 msec. When the prime stimulus was re-illuminated (for 100 msec) the monkey had

to respond (within 650 msec) according to two task conditions (or rules). In the *compatible* condition the animal had to reach the prime stimulus location, whereas in the *incompatible* condition the monkey always had to move toward a fixed location. Because the animal was forced to continue looking at the central location and either remember or attend the location of the prime stimulus until the beginning of movement, this experimental design allowed us to test the hypothesis that neuronal activity reflects the motor instructional significance of the sensory events. If the activity of the cell differed between the two conditions, we could conclude that that neuron's activity was not confined to the sensory aspects of the stimuli or attentional aspects of the task, and must reflect some part of the process by which the stimuli are used to select and trigger actions. Current views on the functional organization lead to different predictions for the behavior of cells in PM vs. PF, and we recorded from populations of neurons in both areas.

Subproject #13. Two monkeys were trained to depress a switch and to fixate a visual stimulus (the fixation point). A second stimulus then appeared somewhere in the visual field. When that second stimulus dimmed, the monkey had to release the switch to receive a reward. By varying the locations of the fixation point we could examine the coordinate system for the apparent visual responses in PM. It has been known since the initial studies on this project that there appear to be neurons in PM with visual responses, but little is known about this pattern of activity. It has been reported (Gentilucci et al., Exp. Brain Res. 50: 464; 1983) that these cells have receptive fields that are independent of gaze. I.e., these investigators claim that each cell is excited by visual stimuli in a certain position relative to the animal's head or body rather than by its position on the retina. This observation, if true, would be of immense theoretical significance for understanding the mechanisms by which movement direction is encoded in the frontal cortex. Unfortunately, from a methodological perspective, the report of gaze-independent receptive fields in PM by Gentilucci et al. was unsatisfactory, and the issue required rigorous investigation under controlled experimental conditions.

Subproject #14. We conditioned two rhesus monkeys to perform a complex visuomotor task designed to have both convergence and divergence in stimulus-movement relationships. In a convergent relationship, two or more different stimuli instruct the same action. By contrast, in a divergent relationship one stimulus instructed two or more different actions. To implement these experimental designs we trained the monkeys to maintain fixation on a target while they attend selectively to other stimuli and later prepare a limb movement on the basis of those stimuli. First, a cue instructed the monkey to either remember or attend to one of several positions in visual space. This cue was termed the "spatial attentional/mnemonic cue." Later, a second stimulus was presented, one which instructed the monkey about what forelimb movement to make on that trial. The second cue, termed the "motor instruction/conditional cue", could consists of either one or two colored squares. If there were two squares, one was red and the other green and only one of these was relevant — the one that appeared at the same location in space as had the spatial attentional/mnemonic cue. If the relevant stimulus was green, the monkey had to move its limb to the right, but if that stimulus was red, the monkey had to move left. This experiment was designed to study the relationship between PM and PF cells reflecting motor preparation (intention) vs. selective attention. We could also examine the possibility that the activity in these cortical regions reflect visual responses vs. motor preparation. We could test these possibilities by two divergent relationships inherent in our experimental design: (1) we could contrast the "response" to a physically and spatially identical stimulus when it instructed the animal to attend to or remember a spatial position vs. when it instructed a movement and (2) we could compare putative responses to identical stimuli when, depending on the prior location of the spatial attentional/mnemonic cue, they instructed oppositely directed limb movements. In additional, we could compare neuronal activity in the convergent relationship, i.e., when different stimuli instructed the same response. We predicted that PM neurons would be dramatically

affected by the movement rather than solely by the stimulus, even when the timing of discharge presented the *prima facie* appearance of "sensory" responsiveness.

MAJOR FINDINGS:

Premotor Cortex Physiology

In general terms, we have adopted three experimental approaches to distinguishing activity related to sensation and perception from that reflecting the selection and control of motor behavior: (1) convergent designs, in which two or more stimuli instruct the same actions; (2) divergent designs, in which one stimuli instructs two or more actions; and (3) parallel designs, in which a stimulus instructs a single response, but under two circumstances: one involving the selection of movement based on arbitrary stimulus-response associations and the other involving the selection of the same movement on some other basis.

In our initial studies (subprojects #1 to #9), we described four patterns of neuronal discharge during what has come to be called an instructed delay period. We termed these patterns, movement-related activity, set-related activity, signal-related activity, and anticipatory (or pre-cue) activity. We hypothesized that PM discharge, especially the set-related activity, is specifically correlated with the motor preparation (or "set") of an animal. Our initial support for this hypothesis consisted of the following findings and observations: (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; (b) if the visual instruction changes (to establish a different motor set), the unit activity rapidly changes to reflect the new set; (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; (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; (e) set-related activity is usually the same when directional (left or right) instruction stimuli and arbitrary (yellow or blue) instruction stimuli instruct the same movement; (f) set-related activity is usually the same when the monkey plans a movement on the basis of trial-specific visual stimuli and when the monkey plans the same movement on the basis of the memory of recent events; and (g) set-related activity reflects aspects of movement detail rather than a final target's net distance and direction .

In our most recent subprojects, we have found that some of the set-related activity in PM is learning dependent (subproject #11). It has been proposed that PM plays a role in the selection of motor programs based on environmental context. To test this hypothesis we recorded the activity of single neurons as monkeys learned visuomotor associations. The hypothesis predicts that task-related premotor cortical activity before learning should differ from that afterward. We found that a substantial population of premotor cortex neurons, over half of those adequately tested, showed the predicted learning-dependent changes in activity. Learning-dependent changes were found for movement-related, set-related, and signal-related activity, as well as for anticipatory activity. The findings of subproject 11 support a role for PM in motor preparation, generally, and suggest a specific role in the selection of movements on the basis of arbitrary associations. 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. Our most recent findings on this subproject, although preliminary at this time, are that the supplementary motor cortex shows greater activity earlier in learning novel stimulus-response associations.

Subproject #10 determined that the reported physiological distinctions between PF and PM reflect *bona fide* differences rather than technical considerations such as differences among

species, individuals, or behavioral, physiological, and anatomical techniques. PM and PF cortex differ in several respects. Cells with apparent selectivity for stimulus characteristics appear only in PF. During the instructed delay period, cells in PF begin activity earlier than PM neurons, whereas PM neurons continue their activity longer. Similarly, PF cells show deeper discharge modulation early in the instructed delay period compared to PM cells, which discharge more intensely later. Thus, our results support the view that PM and PF have distinctive physiological properties that cannot be accounted for by technical considerations alone.

Subproject #12 led to a dramatic result: most cells in PM, but relatively few in PF, are influenced by the motor significance of spatial sensory cues. In this behavioral paradigm, the monkey was required to attend to and respond to spatial sensory cues in two conditions. In one condition, the cues instructed the monkey about the direction of limb movement to execute on that trial. In the other, the cues indicated only *when* the movement was to be executed, and the movement was the same on all trials. The stimuli fell on the same part of the retina (and in all other spatial coordinate systems) and had to be attended to in the same way. In PF most cells showed the same activity during both conditions, indicating that the motor significance of the stimuli did not affect its activity and that the cells were most likely involved mainly in sensory information processing and perception. In PM, by contrast, most cells were dramatically affected by the motor significance of the stimuli, supporting the view that they play their most significant role in the selection and control of motor behavior.

Subproject #13 was prompted by a report from another laboratory which suggested that cells in PM have a constant spatial response regardless of gaze angle. Such a property would have been the first instance of "gaze-independent" receptive fields anywhere in the visuomotor system and, as such, would have been of considerable theoretical importance. Such properties would simplify greatly the challenge of understanding how movements are made to visual targets despite differences in locus of fixation and the initial position of the limbs and body. However, our examination, which was conducted under rigorously controlled conditions, found few, if any, cells with gaze independent receptive fields in PM. Indeed, without clear exception, the response to visual stimuli of every cell studied was affected, usually dramatically, by the position of the eye in the orbit.

Subproject #14. Our results show that the vast majority of cells in the dorsal part of PM have greater activity following intentional cues than after attentional cues. In most instances, there is no activity following attentional shift cues, although the identical stimulus causes profound modulation when presented as a movement instruction. In PF, by contrast, more cells showed comparable or greater activity following the attentional and intentional cues. Further, a majority of dorsal PM neurons, but only a minority of PF neurons, have activity that depends on the motor significance of an identical stimulus. Thus, PM activity during reflects the direction of the upcoming movement and appears to code for aspects of action rather than the sensory signals that guide the act. By contrast, cells in PF appear to have activity more closely linked to the instructing stimuli. Cells in the ventral part of PM appear to have intermediate properties.

The past year's progress on each subproject was, as follows:

Subproject #10. The data analysis was completed and the paper was published in the journal *Brain*.

Subproject #11. Data from the first aspect of this subproject was analyzed and published in the *Journal of Neuroscience*. Data collection on the second phase of the project, which is meant to contrast the activity of PM and the supplementary motor area has begun and continues.

Subproject #12. The data collection phases of this subproject were completed and data analysis is nearly done. A paper concerning the results of this subproject was presented at the annual

meeting of the Society for Neuroscience in November and a full-length paper on the subject was submitted for publication in June to the *Journal of Neuroscience*. An especially interesting aspect of this subproject has been combined with data from subproject #14 and was submitted for publication to the *Journal of Neurophysiology* this May.

Subproject #13. Data collection for this subproject was completed and the data analysis performed. An abstract concerning the preliminary results of this subproject will be presented at the annual meeting of the European Neuroscience Association this September and a full-length paper has been submitted for publication to the journal *Experimental Brain Research* this April..

Subproject #14. Data collection was completed and data analysis in ongoing. The initial aspects of the results have been joined with one part of the results on subproject #12 for submission to the *Journal of Neurophysiology* in May and the major part of the results were submitted for publication to the journal *Experimental Brain Research* in July.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE:
This project is devoted to studying the biology of the frontal cortex, which plays an important role in higher brain functions such as intention, attention, and learning. Functional localization in higher-order cortical fields remains poorly understood, although there is increasing recognition that diseases such as schizophrenia, attention deficit disorder (ADD), obsessive compulsive disorder (OCD), panic and mood disorders, and a variety of dementias, including Alzheimer's, result at least in part from frontal lobe dysfunction.

PROPOSED COURSE OF THE PROJECT:
Behavioral Neurophysiology
In future years, the motor learning project (subproject #11) will be elaborated to include investigation of other kinds of motor learning. The type of motor learning we have studied is termed conditional motor learning, which involves learning to execute a specific motor act in response to an arbitrary stimulus. Other types of motor learning include the acquisition of a motor skill and learning a sequence of motor acts. We plan to compare PM activity during different kinds of motor learning. In addition, the oculomotor system offers many technical and methodological advantages in the study of the cerebral control of movement. We are currently training a monkey to perform an oculomotor version of subproject #11, and expect to begin data collection from this animal in this fiscal year.

Dr. Donald Crammond will join the LNP this fiscal year and embark on an extension of the LNPs work on PM and the signals that are involved in the preparation of limb movements. In addition, a prime focus of Dr. Crammond's work will involve an attempt to relate slow-wave, electroencephalographic activity, such as that commonly studied in human and nonhuman primates, to the single neuron activity that has been the focus of so much investigation in monkeys. This aspect of the study will be undertaken in collaboration with the NINDS Human Motor Control Section (Dr. Mark Hallett, Chief), who will be conducting parallel studies in humans with a special emphasis on the localization of the dipoles that generate the slow wave potentials preceding movement. This collaborative effort promises substantial progress in bridging the gap between basic and clinical studies on the brain signals that generate movements.

Neural Telemetry and Vocalization
We are continuing our attempt to develop telemetric recording of single-cell activity in the primate frontal cortex. As successful as behavioral neurophysiology has been, the method suffers from a fundamental limitation: the behavioral environment is radically divorced from the normal life history of the animal. For the purpose of controlling variables and for technical reasons as well, monkeys are placed in situations which are highly unnatural. This approach has been led to

substantial progress in understand neural networks related to motor control and sensory perception, but there is a need to study the neural basis of behavior in an ethologically more meaningful context. Much of primate behavior consists of social interactions. Of special interest to neurophysiologists is species-typical communication, which is very sophisticated and moderately well studied, at least in certain primate species. Current methods in behavioral neurophysiology do not allow sufficient flexibility to establish ethologically meaningful environmental conditions. We hope the establishment of reliable methods for telemetric recording of single cell activity in the central nervous system will lead to a new perspective on the behavioral neurophysiology of communicatory systems.

A detailed prospectus of this technical development subproject was prepared by Dr. Mitz and distributed to experts in the field and at NIH. The comments of these experts were considered and an initial system design decided upon. There are four main problems to be overcome: (1) signal transmission, (2) electrode mobility and stability, (3) single-neuron isolation, and (4) experimental design. This year has seen the completion of the first prototype telemetry device and we will soon begin its testing. The initial tests on transmitting neural signals from chaired monkeys has been successful.

Our initial project will involve a study of functional specializations of different cortical areas underlying vocalization in primates. There is reasonable evidence that some frontal agranular areas in and near the cingulate sulcus and other areas near the frontal operculum play a role in the control of vocalizations. Lateral frontal areas may play an important role, as well. Since vocalization is a motor output that can be easily and rigorously measured, and also one which has an undoubted ethological significance, we believe that it will serve as the best starting point. One way to trigger a vocalization in some monkeys is to isolate them from their social group. Following such isolation, they spontaneously emit the motor act, i.e., the vocalization. Alternatively, the behavior can be brought under stimulus control, either via arbitrary, operantly conditioned stimulus-response associations or as responses to the recorded natural vocalization of conspecifics. We propose to examine the hypothesis that separate neuronal pathways subserve triggering of a behavior by these different conditions and stimuli. More specifically, we intend to test the hypothesis that medially situated "vocalization areas" play a role in internally or self-generate vocalization, such as those that occur upon isolation from conspecifics, whereas laterally situated areas play a role in operantly conditioned vocal output.

PUBLICATIONS:

Journal Articles

Di Pellegrino G, Wise SP. A neurophysiological comparison of three distinct regions of the primate frontal lobe, Brain 1991;114:951-978.

Mitz AR, Godschalk M, Wise SP. Learning-dependent neuronal activity in the premotor cortex of rhesus monkeys, Journal of Neuroscience 1991;11:1855-1872.

Book Chapters

Houk JC, Wise, SP. Outline for a theory of motor behavior: Cooperative actions of the cerebral cortex, the basal ganglia, and the cerebellum. In: Roudomin P, ed. Models of the Motor System. New York: Raven Press, 1992 (in press).

Wiesendanger M, Wise SP. Current issues concerning the functional organization of the primate motor cortex. In: Chauvel P, Delgado-Escueta AV, Halgren E, Bancaud J, eds. Frontal Lobe Seizures and Epilepsies. New York: Raven Press, 1991;117-134.

Wise SP. A brief history of the basal ganglia. In: Carrol BJ, Barret JE, eds. Psychopathology and the Brain. New York: Raven Press, 1991;45-61.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER:

ZO1 MH 01098-06 LNP

PERIOD COVERED

October 1, 1991 to September 30, 1992

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 Senior Staff Fellow LNP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Animal Center, Poolesville, Maryland 20837

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

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

This project seeks to improve our understanding of the detailed circuitry of the thalamus and its relation to arousal, sleep-wakefulness cycles, and information transfer from subcortical relays to the cerebral cortex. Recent evidence suggests that the reticular nucleus of the thalamus (TRN) participates in the generation of the high-voltage rhythmic activity in the forebrain during slow-wave sleep. The focus of this study is to identify the afferent inputs which might influence the excitability of neurons within the TRN and to determine the details of the relationship of these afferents to the cell bodies and dendrites of TRN neurons.

During the past year, the technique of photoconverting intracellularly injected Lucifer yellow dye within neurons in order to make the label electron-dense, and the subsequent processing of tissue for electron microscopic (EM) analysis was perfected, and identified TRN neurons were examined at the EM level with the following goals: (a) to identify the synapses upon different segments of the dendrites of individual TRN neurons, and (b) to determine whether or not some of the finer beaded 'dendrites' of these neurons might give rise to dendrodendritic synapses which could serve to coordinate the activity of neighboring TRN neurons. When identified TRN neurons are examined in the EM, both asymmetric and symmetric synapses are observed upon all dendrites and the density of the synapses is relatively constant along the length of the dendrites. Occasionl dendritic branch points, however, appear to be densely innervated by asymmetric synapses whose pre-synaptic element may correspond to D-type terminals. Synaptic contacts were observed upon the swollen, as well as the constricted segments of the beaded dendrites, and the fine beaded dendrites were not found to give rise to pre-synaptic profiles.

Objectives:

This project seeks to elucidate the structural details of some neuronal circuits which may be involved in the modulation of signals flowing through the dorsal thalamus to reach the cerebral cortex. While the modulation of thalamic states by cholinergic input systems has received considerable attention thus far, it is important to obtain an understanding of each of the other input pathways which may be involved as well. This project seeks to: (a) identify the sources of afferent inputs to the thalamic reticular nucleus; (b) determine the details of the afferent arborizations with the cell bodies and dendrites of reticular nucleus neurons; (c) test for morphological evidence regarding the transmitters utilized by the neurons giving rise to the afferent inputs; (d) examine the details of reticular neuron interactions with other reticular neurons as well as with its dorsal thalamic and cortical inputs; and (e) identify potential regional sub-specializations within the reticular nucleus. These issues are of interest since virtually all information which enter the cerebral cortex is relayed through the thalamus, and since recent studies have shown that the thalamic reticular nucleus is likely to be a critical node in the thalamic gating circuitry responsible for the rhythmic high voltage spindles recorded in the forebrain and diencephalon during slow-wave sleep.

Methods:

This project has been carried out mainly in rats, although occasional guinea pigs were also used during this past year. The procedures used include: (*i*) anterograde and retrograde neuroanatomical labeling techniques, (*ii*) the intracellular injection of the fluorescent dye, Lucifer yellow, into identified neurons in brain slices, (*iii*) immunohistochemistry, and (*iv*) electron microscopy. The specific procedures for each are outlined below.

(*i*) For the anterograde and retrograde neuroanatomical experiments, injections of tracers are made into various brain foci. Following appropriate survival periods, the animals are perfused with heparinized saline followed by a buffered aldehyde fixative solution, and the brains sectioned on a vibratome or on a freezing microtome. The appropriate histological procedures are performed, and sections from selected regions are examined microscopically using both low power objectives and high power oil immersion objectives. Data are documented with photomicrographs or with camera lucida drawings.

(*ii*) In those experiments involving the intracellular Lucifer yellow injections, the brains are sectioned on a vibratome, counterstained with nuclear yellow, secured on glass slides with a nitrocellulose membrane filter, and examined using a fluorescent microscope, equipped with a high power (40x) water immersion objective, with 1.6 mm working distance. Neurons in the thalamic reticular nucleus are identified and impaled under visual guidance with glass microelectrodes introduced at an angle of 10-30 degrees from horizontal, and containing a 5% (w/v) solution of Lucifer yellow. As the dye is iontophoresed into the neurons, it quickly diffuses throughout their dendritic arbors. Following the injection procedure, the sections are removed from the membrane filter, briefly postfixed in 4% formaldehyde, and, when appropriate, run through standard immunohistochemical procedures. The sections are then mounted on slides and prepared for microscopic examination and documentation as briefly outlined above.

(*iii*) For the immunohistochemical experiments, following the appropriate fixation, tissue sections are cut and incubated in a primary antibody solution, followed by incubation in a secondary antibody solution. The secondary antibody used is generally conjugated to a fluorescent compound or subsequently bound to the enzyme, horseradish peroxidase, and the tissue is reacted for peroxidase immunohistochemistry. The sections are then mounted, and coverslipped, and regions of interest, examined and documented.

(*iv*) For the electron microscopic experiments, procedures (*i*) and (*ii*) are performed with minor modifications. The tissue blocks containing Lucifer yellow injected cells are bathed in a 1.5% DAB solution and excited with a UV light transmitted through a Nikon B-3A filter system, and are subsequently excised and postfixed in osmium, stained en bloc in uranyl acetate, dehydrated through a graded series of alcohols, and flat embedded in plastic. Ultrathin sections are cut, serial thin sections mounted on grids, stained with lead citrate, examined, and photographed in a Zeiss EM-10.

Major Findings:

During this past year, I have focussed upon objectives (d) and (e) outlined above. Towards a better understanding of regional subspecializations within the thalamic reticular nucleus, I have examined the reticular nucleus of guinea pigs, which unlike many other mammals, is distinctly laminated in its rostral sector (rostral 2/3 or so). By using standard nissl and cholinesterase staining, it became apparent that the rostral reticular nucleus of guinea pigs is highly differentiated. Although differentiation had been proposed in the caudal reticular nucleus of *Galago senegalensis* (Conley et al., Eur.J.Nsci. 3:237,1991), the part proposed to be distinct (a thin medial wedge) was later shown instead to be a rostral extension of the ventral lateral geniculate nucleus (Conley et al., J.C.N. *in press*). However, lamination of cortical inputs within the reticular nucleus has been noted recently by several groups of investigators (Crabtree & Killackey, Eur.J.Nsci. 1,:94,1989, Conley & Diamond, Eur.J.Nsci. 2:211, 1990), thus, I examined the possibility that there might be distinct subdivisions within the markedly laminated rostral portion of the main reticular nucleus of guinea pigs by applying many of the standard neuroanatomical markers used in identifying thalamic nuclei. Towards this goal, a gamut of immunohistochemical staining was performed. These include staining for: GABA, GAD, ChAT, serotonin, somatostatin, parvalbumin, calbindin, and DBH. In brief, the results of these immunohistochemical procedures indicate that although seemingly bilaminar upon casual inspection, the staining patterns throughout the width of the rostral reticular nucleus are quite similar following all of the immunohistochemical procedures which were performed. It remains possible, however, that connectional differences as well as morphological differences among the cells themselves might underlie the reticular nucleus lamination seen with nissl staining and cholinesterase staining guinea pigs, and it may be worthwhile to pursue this at some future date.

The morphology of the thalamic reticular nucleus has been investigated by a number of investigators in rats and higher mammals (see eg. Ramón y Cajal, Histologie du Système Nerveux, Maloine, Paris, 1909-11, Scheibel & Scheibel, B.R. 1:43, 1966, Jones, J.C.N. 162:285, 1975, Spreafico, J.C.N. 304: 478, 1991). In all species examined, the general appearance of thalamic reticular nucleus neurons is quite similar. TRN neurons have sparsely branching dendrites, and their dendrites extend for considerable distances (well over 1 mm in tangential diameter in most parts of the nucleus vs. at most, only a couple of hundred microns in diameter for dorsal thalamic neurons) within the tangential plane of the reticular nucleus. All neurons within the reticular nucleus are GABAergic, and the dendrites of these neurons are very sparsely spined (in adult animals), though highly beaded.

Although information is available on the morphology of synaptic terminals corresponding to each of the major input pathways entering this thalamic nucleus, little is currently known about the precise distribution of these terminals upon individual reticular nucleus neurons. Dendritic diameter, a parameter commonly used in thin sections to determine how far or near to the neuronal cell body the dendrite lies, can be misleading when applied to some of the smaller dendritic processes of reticular neuron dendrites. These dendrites are highly beaded, and the dendritic branches which emerge from a given branch point are frequently of unequal size. While most of the primary and secondary dendrites of reticular nucleus neurons are quite thick proximally, and taper gradually away from the cell body, fine dendrites, whose hair-like stems remain constant in diameter for considerable distances, are also frequently observed to emerge directly from the somata or large primary dendrites. Thus a synapse apposed to a small dendritic profile may not be upon a distal dendritic segment, but rather may actually occupy a constricted dendritic segment of a fine dendritic branch emerging close to the cell body.

In January of 1991, a Zeiss EM-10 unit was transferred to the LNP from the CBDB at the NCSE. During this past year I have concentrated on working out details of the procedures necessary to obtain high quality electron microscopic preparations (eg. identifying each of the multitude of sources of contamination, etc.). As an initial electron microscopic project, I have begun to examine the details of the terminals which synapse upon different segments of individual reticular nucleus dendrites as well as to determine whether or not some of the highly beaded, fine caliber dendritic processes are presynaptic to other dendrites. Towards this goal, I have injected Lucifer yellow intracellularly into reticular nucleus neurons in the lightly fixed slice preparation, photoconverted the fluorescent marker into an electron-dense reaction product by bathing the tissue in DAB in the presence of intense UV light, osmicated the tissue, stained the tissue 'en block' in uranyl acetate, then dehydrated and embedded the tissue in epon. From these tissue blocks, serial thin sections were cut, stained with lead citrate, and examined in the EM.

When filled with Lucifer yellow and photoconverted, several features of reticular nucleus neurons are apparent. Dendritic branches always emerge from enlarged segments of the beads, and the branches never arise from the constricted dendritic stems. The fine beaded dendrites are frequently observed near the terminal segments of reticular neuron dendrites, but they occasionally emerge from the cell bodies or directly from one of the thick primary dendrites. When tissue slabs containing intracellularly filled neurons are taken to the EM level, the labeled neurons and their processes can be readily identified within the neuropil. A dark DAB reaction product is distributed throughout the dendritic matrix of these neurons, however, the subsynaptic membrane densities can be seen quite readily through the reaction product at sites of receipt of synaptic contact, and asymmetric and symmetric synaptic contacts can be identified quite readily. The primary stem dendrites are in receipt of numerous synapses; both asymmetric and symmetric synapses are identified upon these large dendrites. Near dendritic branch points, there is occasionally an increased incidence of asymmetric synapses. These generally tend to occur on the beaded enlargements from which the branches emerge, and the pre-synaptic profiles appear to correspond to D-type terminals. Occasionally, a high concentration of D-like terminals are evident as well on the proximal stretch of one, or both of the daughter dendrites.

Two fine dendrites which emerge from primary dendrites relatively close to the cell body were identified and examined in detail. These fine dendrites had stem diameters of $\approx$ 0.2 μm, with $\approx$ 0.5 X $\approx$1.2 μm enlargements at periodic intervals, quite comparable in dimensions to many unlabeled axonal profiles scattered throughout the neuropil and to the dimensions of the axonal terminals

of these neurons previously examined within the dorsal thalamus (Montero, Nsci., 6:561, 1981, Cucchiaro et al., J.C.N. 310:316, 1991). These dendritic processes were followed from their point of emergence distally for stretches of 40 µm and 70 µm respectively in serial thin sections. Unlike some of the larger collaterals, there were no pronounced accumulations of asymmetric synapses upon the proximal segments of these fine dendrites. Instead, the density of synapses upon the proximal segments of these fine dendrites was comparable to the density seen on distal segments. The small beads belonging to these fine dendrites received, on average, 2 - 3 synapses, and both asymmetric and symmetric synapses were identified. The thread-like constricted segments received synapses as well, although their occurence was less frequent, and many segments appeared not to be in receipt of any identifiable synapses. Both symmetric and asymmetric synapses were identified upon the constricted segments. The dark DAB reaction product within the dendritic matrix obscured the organelles within, and it was difficult to identify the presence/absence of vesicles within. The relation of these fine dendrites to adjoining profiles was, however, scrutinized carefully. Throughout the lengths which were followed and analyzed, these fine dendrites could not be identified to comprise the pre-synaptic element at any identifiable synaptic junction. These processes were consistently post-synaptic.

These preliminary observations indicate that the density of synapses upon reticular neuron dendrites remain relatively constant along the length of the dendrites, but that higher concentrations of probable D-type terminals occur in close proximity to some dendritic branch points. These observations also indicate that dendrodendritic synapses if at all present are quite uncommon in the rat reticular nucleus. Since in most cases, it is difficult to be certain that the neurons are completely filled, it remains possible that synaptic interactions among reticular neurons dendrites might occur further distally. However, it seems probable that the majority of dendrites of reticular nucleus neurons in the rat appear to be exclusively in receipt of synapses, and thus it is likely that potential interactions among reticular nucleus neurons takes place via local axonal collaterals of reticular nucleus neurons rather than through dendrodendritic synapses in this species.

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

Nearly 50 years ago, Morison and Basset (*J. Neurophysiol.* 8:309, 1945) hypothesized that the thalamus generates the rhythmic 7- to 14 Hz spindle oscillations characteristic of EEG-synchronized sleep and that these oscillations are somehow suppressed during "activated" states such as arousal and REM sleep. Interest associated with these rhythms has recently focused on the reticular nucleus of the thalamus, since the integrity of this nucleus appears to be critical for the occurence of the rhythmic oscillations underlying the spindle sequences of synchronized sleep (Steriade & Llinás, *Physiol. Rev.* 68: 649, 1988). I have previously focused upon the structural details of some of the 'diffuse' extrinsic inputs which innervate the reticular nucleus, since these are likely to be involved in influencing the excitability of reticular nucleus neurons. The identification of an extrinsic GABAergic input is of interest not only towards our understanding of the factors influencing reticularis-mediated state-dependent thalamic gating, but towards a better understanding of functions impaired in the progression of Alzheimer's dementia. The dense, direct noradrenergic innervation of reticular neuron dendrites indicate an important role of the ascending noradrenergic system as well upon the discharge states of reticular nucleus neurons. This projection may be of relevance to the disturbances of sleep and vigilance associated with many psychoses. While a complete understanding of all the factors influencing sleep and arousal and the elucidation of the neural mechanisms which bring about these different brain states remain quite distant, there is much that can be learned, with the techniques which are presently

available, about the details of the anatomical circuits which may be critically involved in thalamic gating mechanisms. A systematic ultrastructural analysis of the reticular nucleus and the intralaminar nuclei in the rat thalamus, and comparisons to the organization of these nuclei in higher mammals, provide an important structural foundation towards our eventual understanding of the role played by these thalamic centers in influencing forebrain activity.

Proposed Course of the Project:

In the immediate future, I plan to investigate further, the details of some of the circuits which potentially participate in thalamic gating mechanisms. In particular, I plan to pursue the following:

(a) Synaptic interrelations among the dendritic arbors of reticular nucleus neurons, as well as the precise distribution of corticothalamic and basal forebrain inputs upon identified reticular nucleus neurons, in rats and monkeys. First, I hope to increase my sample size of recovered reticular nucleus neurons in rats, to be more confident of the preliminary observations I have made. Beyond this, however, there are at least two relatively straightforward labeling experiments I would like to do in conjunction with the EM analysis of identified reticular nucleus neurons. Previous electron microscopic studies have concluded that corticothalamic terminals as well as the cholinergic terminals within the thalamic reticular nucleus neuropil are likely to correspond to the D-type terminals. Whether or not there is a differential distribution of these two inputs upon individual reticular nucleus neurons would be of much interest. Towards this goal, I plan to lesion the rostral cortex, and subsequently carry out the procedures I've worked out for identifying individual TRN neurons in the EM to identify the precise locale of the degenerating synapses. A similar procedure would be followed for the inputs from the basal forebrain. Which source might give rise to the occasional dense aggregations of D-type terminals at dendritic branch points would be of much interest. Although I have previously examined the projection onto the thalamic reticular nucleus from the caudalmost portion of the basal forebrain, the observations were limited to axons arising in the caudalmost sector of the magnocellular basal nucleus, and the projections from more rostral basal forebrain areas such as the horizontal and vertical limbs of the diagonal band have yet to be examined. Preliminary experiments using PHA-L at the light microscopic level indicate that the bouton-like enlargements associated with axons arising in these more rostral parts of the basal forebrain are of finer caliber than those arising caudally. This suggests that these might correspond more closely with the small ChAT-positive boutons previously identified within this nucleus. Thus, a comparison of rostral basal forebrain projections with the distribution of corticothalamic terminals upon identified thalamic reticular neuron dendritic arbors would be of interest. I would also like to extend this project to monkeys, since dendrodendritic synapses within the TRN have been suggested to be more prevalent in higher mammals. The precise locale of potential interactions among the dendritic arbors of TRN neurons is of considerable interest.

(b) The ultrastructure of brainstem and TRN afferents upon the intralaminar nuclei in rats and monkeys. This long-range electron microscopic study seeks to identify the ultrastructure of afferents from the peribrachial brainstem region (the parabrachial nucleus, the laterodorsal tegmental nucleus, and the pedunculopontine nucleus) within the intralaminar nuclei, as well as to identify its relation to projection neurons and to GABAergic local thalamic interneurons (in monkeys). Physiological evidence accrued over the last 50 years or so indicates that the intralaminar region of the thalamus is likely

to be involved in mediating the desynchronization of the cortex during arousal. Although retrograde transport experiments which have combined retrograde tracer transport and immunohistochemistry have identified cholinergic neurons within the peribrachial brainstem region projecting upon the thalamus, it is apparent that not all neurons within these regions projecting upon the thalamus are cholinergic. The ultrastructure of the axonal terminals arising in the brainstem is, at present, unclear, as is their relation to the neurons within the intralaminar region. It would, therefore, be of interest to identify the cell types in receipt of synapses from these brainstem cell groups. I would like to extend this project to monkeys as well, since GABAergic thalamic interneurons are most prominent in monkeys, and whether the brainstem inputs might directly contact thalamic interneurons as well as the principal neurons would be important in terms of understanding thalamic information processing. Later, I would like to extend this project to include a comparison of brainstem inputs with reticular neuron inputs upon identified neurons in this region.

Publications:

Journal Articles

Asanuma C. Mapping movements within a moving motor map, *Trends in Neuroscience* 1991;14:217-218.

Asanuma C. Noradrenergic innervation of the thalamic reticular nucleus: A light and electron microscopic immunohistochemical study in rats, *Journal of Comparative Neurology* 1992;319:299-311.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER:

Z01 MH 01101-01 LNP

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Cortical Mechanisms of Auditory Processing in Cats and Primates

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	.25

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

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

This project seeks to improve our understanding of the neural basis for acoustic communication in the auditory cortex of higher mammals. Compared to visual and somatosensory cortex, our knowledge about the organization of auditory cortical areas is much poorer, although they are highly important for the processing of acoustic communication signals. We have started a new attempt to analyze the neural code for auditory processing in nonprimary auditory cortex. In addition to pure tones, standardized complex sounds were used for the stimulation of single neurons. Frequency-modulated (FM) sounds, as they occur in many natural vocalizations, were generated on a digital computer and presented to neurons in the anterior auditory field (AAF) in cats. Complex quasi-natural sounds were also recorded digitally and played back for stimulation. Neurons in AAF responded best to highly transient stimuli with a fast rate of frequency modulation, and AAF may therefore be involved in the processing of spatial aspects in audition.

In macaque monkeys, the same approach was taken to explore the multiple auditory maps in nonprimary auditory cortex. Neurons in the rostro-lateral area (RL) responded preferentially to low-frequency sounds, as they are contained in communication signals. By contrast, neurons in the caudo-medial area (CM) preferred high frequencies, which are important for sound localization. Lesioning of primary auditory cortex (AI) seemed to affect responses in area CM more than it did in area RL, which could be due to the different input of these two areas from the medial geniculate nucleus (MGB) of thalamus. CM may depend more on input from AI, while RL (like AI) receives direct input from the parvocellular layers of MGB.

Objectives:

One of the great revelations in our understanding of the visual cortex over the past two decades has been that there exist a multiplicity of different representations of the visual world. Each of these maps seems to be specialized for a certain aspect of visual processing, such as stereopsis, color or motion. It appears very likely that there is also a multiplicity of representations in the auditory cortex. Both from cytoarchitectonic and from physiological mapping studies at least four different subfields (probably more) have been identified around what is known to be primary auditory cortex in cats and monkeys. Except in bats, however, very little is known about the functional specializations of these subfields. In this project, we are exploring nonprimary auditory cortex physiologically with standardized stimuli that are generated on a digital computer and contain important components of natural vocalizations. At a later stage we plan to combine this approach with anatomical tracer studies, in order to characterize the input-output connections of the cortical maps and relate them to their physiological response properties.

Methods:

Non-primary auditory cortex was explored with extracellular microelectrode recording in cats and monkeys. Complex auditory stimuli were designed on a 486 computer using the SIGNAL program package and played back to the animals during unit recording. These stimuli included frequency-modulated (FM) sounds, as they occur in many vocalizations, as well as standardized environmental sounds. Peri-stimulus time histograms (PSTH's) and raster displays were used to quantify the responses. In macaque monkeys, the effect of primary auditory cortex lesions on neuronal responses in nonprimary areas was tested in addition to their normal functional organization.

Major Findings:

A. Auditory Processing in the Cat's Anterior Auditory Cortex

The single most important way to characterize multiple representations in the cortex is by means of stimulus preference of their neurons. We believe that a lot can be learned about auditory cortex by analogy with the visual system. It is clear that cochleotopy, as defined from stimulation with pure tones, exists in auditory cortical areas in the same way as retinotopy exists in the visual cortex. However, more complex stimuli than pure tones have to be used for functional mapping of response properties. Frequency-modulated (FM) sounds are among the simplest of complex sounds and are interesting for two reasons. First, they occur in a great number of natural vocalizations of many different species, including man. In addition, FM sounds have the intriguing property that, with regard to the receptor surface, they are equivalent to moving light stimuli in the visual system, which are known to drive most cells in the higher visual pathways most effectively.

FM sounds were used for stimulation of single neurons in two anterior regions of nonprimary auditory cortex, the anterior auditory field (AAF) and the auditory field in the anterior ectosylvian cortex (AEA). Both areas were found to have a preference for fast changes in the frequency domain. Almost one half of the neurons showed high-pass properties for the rate of frequency modulation. The other half responded best to a

certain narrow range of modulation rates. The instantaneous frequency at the response peak generally matched the best frequency in the pure-tone tuning curve. However, one third of the neurons responded with multiple peaks when tested with FM sweeps, while most of them were broad-band tuned with a single peak when tested with tone bursts. Two thirds of the neurons in AAF had a preference for one direction of the FM sweep over the other, when a 50%-criterion was applied to the difference in both responses.

Various other complex stimuli were also used, which were derived from natural sounds by recording these sounds on a digital computer and playing them back to the neurons in a standardized way. Again, neurons in the anterior auditory regions preferred usually the most transient of these natural complex sounds. Spatial tuning of neurons in both anterior areas was measured in azimuth by presenting stimuli in 7 different positions. About one half of the cells were tuned for spatial location

The anterior auditory region of the cat's cortex therefore seems to specialize in the processing of fast-changing, transient sounds, since neurons in other cortical areas more often prefer slower rates of frequency and amplitude modulation. In addition, it may participate in the processing of auditory space by means of its spatially tuned neurons and its intimate connections with the tectum.

B. Multiple Auditory Representations in Macaque Auditory Cortex

In the visual and somatosensory cortex the flow of information beyond the primary cortical field is organized into two functional streams: one for spatial localization and another for pattern recognition or object identification. While most features of vocalizations clearly fall into the latter category, localization of sound sources is also an important aspect of acoustic communication. However, unlike in the visual or somatosensory system, auditory localization cannot simply be done by using a place code, because information about auditory location is not contained on the receptor surface. Rather, it has to be computed by combining information from the two ears.

A computational map of auditory space is already formed subcortically in the external nucleus of the inferior colliculus (ICX) and in the superior colliculus. Interestingly, these nuclei appear to project to the auditory cortex in a separate pathway through the magnocellular portion of the medial geniculate. Little data are available at present how the auditory spatial information is distributed from there. It is conceivable that, like in the other sensory systems, spatial information is kept separate from pattern information in two different pathways, and that information from different modalities is eventually combined in the same part of the brain: spatial information in parietal cortex and pattern or object information in temporal cortex.

By analogy to the visual system, neurons in the caudo-dorsal pathway should therefore be fairly unspecific for sound frequency, but respond extremely well to rapid FM modulations; in addition, these cells might have good spatial tuning and should be organized in a space map. Neurons in the rostro-ventral part of the auditory pathway by contrast should require rather complex sound patterns (frequency combinations) for an optimal response and might be more sensitive to modulations of sound amplitude (AM) rather than frequency.

In a collaborative project with the Laboratory of Neuropsychology, defined lesions have been made into primary auditory cortex AI, and their effect on higher-order areas (RL and CM, as defined by Merzenich and Brugge, 1973, or paAr and paAc according to Pandya and Sanides, 1973) were assessed by single unit recording. In collaboration with Mort Mishkin's group, we are also planning complementary behavioral experiments to assess the effects of such lesions on auditory perception and memory.

Another approach we have taken to reveal any functional parcellations similar to those that exist in the primary visual areas was to look at the pattern of cytochrome oxidase (CO) staining in AI and its surrounding areas. Only one preliminary study has described intense CO staining of parts of the supratemporal plane, but its functional pattern has not yet been analyzed in detail.

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

Research on the biological basis of language is among the most important, yet highly neglected areas of neurobiology. Research on the organization of nonprimary auditory cortex in higher mammals, especially nonhuman primates, is the main window we have for an understanding of the neurobiological foundation of language. Even though monkeys do not possess the capacity for language in the same way as humans do, evolutionary arguments suggest that precursors of language capacity should exist both in the auditory and in the motor system. Acoustic signals are a rich source of communication for all primate species, and the structure of these communication sounds has been especially well studied in squirrel monkeys and macaques. Our hypothesis is that higher areas of auditory cortex, which are specialized for the processing of these communication sounds, have evolved into areas for language processing in humans. An understanding of the neural code in these higher auditory areas of nonhuman primates should therefore yield valuable information about the neural coding of human speech and language understanding. Unravelling the normal organization of language in the brain will be indispensable for the understanding of higher language disorders, such as Wernicke's aphasia. It is my understanding that up till now such a project has been absent in the NIMH, and we feel, therefore, that we can add a new component to the program of the Institute.

Proposed Cource of the Project:

The next step towards understanding multiple auditory representations will be to delineate them in terms of their input and output connections. Therefore, I plan to make tracer injections (HRP, WGA, fluorescent dyes) into physiologically identified parts of areas AI, RL and CM and look at the connectivity pattern with other cortical areas and with the thalamus. Conversely, injections with anterograde tracers will be made into the different subdivisions of the medial geniculate nucleus (magnocellular vs. parvocellular as well as dorsal vs. ventral), which also need to be explored more thoroughly in functional terms.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER2

ZO1 MH 01102-01 LNP

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Central Auditory Mechanisms for Processing Species-Specific Communication Sounds

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_(b) Human tissues

X (c) Neither

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

The principal aim of this project is to determine the computations and central neural mechanisms for encoding and representing species-specific communication sounds in primates. The squirrel monkey was chosen because of its varied and highly stereotyped repertoire of calls. The experiment adheres to the following strategy: The monkey's calls are analyzed spectrographically to identify their constituent components. A computer is used to model call components and to manipulate these models in the frequency, amplitude and time domains. Modified calls and their components are resynthesized from their models and are used as auditory stimuli to test the responses of neurons in the medial geniculate nucleus (MGN) to frequency ratios and time intervals among call components. This is the first annual report since the project began on June 1, 1991. The principal findings are summarized below.

1. The MGN of the squirrel monkey contains neurons whose responses are facilitated by combinations of components from particular species-specific vocalizations. In this respect, the combination-sensitive neurons in the squirrel monkey resemble those described for lower vertebrates (bats, songbirds, and frogs). Thus, the discovery of combination-sensitive neurons in the squirrel monkey supports the hypothesis that these neurons are of general importance in processing species-specific communincation sounds in vertebrates, including humans.

2. The combination-sensitive neurons in the MGN of the squirrel monkey are sensitive to time intervals (delays) between signal elements. Additional studies are needed to determine the extent to which these neurons are selective for particular vocalizations and whether they are sensitive to frequency ratio or some other spectral characteristic. The delay sensitivity may be a mechanism by which activity in populations of such neurons permit discrimination among acoustically related vocalizations. Similar acoustic discriminations are known to be important in phoneme identification by humans. Thus, we expect that physiological results obtained in the squirrel monkey may provide some insight on the neural mechanisms of human speech perception.

3. Prelimary results suggest that the combination-sensitive neurons are located in the dorsal division of the MGN. Additional studies using physiological mapping and anatomical tracing techniques are needed to determine the functional organization of this region. The expectation is that results from this line of study will lead to a greater understanding of the processing of complex acoustic signals of biological importance in the primate brain.

Background

Computational strategies and neural mechanisms for processing biologically important sounds, including species-specific vocalizations

How the vertebrate auditory system encodes and represents information in biologically important sounds is a central question in neurobiology. The sounds produced and heard by most animals for communication are spectrally complex and are modulated in frequency and amplitude over time to carry information. For humans and for several species of animals it has been shown that sound spectrum and patterns of frequency and amplitude modulation are used to identify and to discriminate among species-specific communication sounds (Brown et al., 1988; Capranica, 1966; Gerhardt, 1978; Liberman et al., 1958; Mattingly and Liberman, 1989; Peterson and Barney, 1952). In these behavioral and psychophysical studies, communication sounds were synthesized from their acoustic components which were modeled by combinations of tones, frequency modulations and noise bursts. Frequency ratios and time intervals between signal components were found to be particularly important for carrying information in vocalizations. For example, psychophysical studies using synthetic speech sounds (phonemes) have shown that the perception of vowel sounds by humans depends primarily on the frequency ratio between tonal components (formants), whereas the perception of many consonant sounds depends on the interval of silence (voice onset time) between a noise burst (plosive) and a vowel sound (Peterson and Barney, 1952; Liberman et al., 1958).

The central auditory sytem of vertebrates has evolved to process information in sounds that are of biological importance to the species. In contrast to neurons at the auditory periphery, many central auditory neurons require stimuli that are spectrally complex and, in some cases, respond selectively to species-specific vocalizations (reviewed by Olsen, 1992; Suga, 1989). One general finding from studies of the bat, the songbird, and the frog is that species-specific vocalizations are processed by neurons that respond selectively to combinations of signal components, and respond poorly, if at all, to individual components of these vocalizations (Margoliash, 1983; Fuzessery and Feng, 1983; Suga et al., 1983; Olsen and Suga, 1991a, b; Sullivan, 1982). The acoustic parameters to which these "combination-sensitive" neurons are tuned, such as frequency ratio and time interval between signal components, are the same parameters that are used by these and other species (including humans) to identify and to discriminate among species-specific vocalizations. These parallels between human speech perception and the processing of complex sound in lower vertebrates suggest that considerable insight into the neural mechanisms of human speech perception may be obtained through a comparative approach.

Neural tuning to frequency ratio or sound spectrum is thought to involve convergence within a tonotopically organized system, such as found in the colliculothalamic projection in the mustached bat (Olsen, 1986; Suga, 1990). It is not known whether this or a similar mechanism operates to produce neural sensitivity to the spectra of communication sounds. Neurons that are tuned to time intervals between acoustic events, such as found in the central auditory systems of the bat and the barn owl, operate by a "coincidence detection" principle: The central auditory system creates transmission delays (delay lines) that delay neurally the activity evoked by one event relative to activity evoked by the event that follows, such that activity from both events are brought to bear at the same place and at the same time (Carr and Konishi, 1988; Olsen and Suga 1991b; Suga, 1990; Suga, Olsen, and Butman, 1990). For the bat, these transmission delays range from one to a few tens of milliseconds, and are produced by excitatory and inhibitory synaptic mechanisms (Olsen and Suga, 1991b; Suga, Olsen and Butman, 1990). A sensitivity to time intervals in the millisecond range has also been implicated in behavioral and psychophysical experiments on the perception of species-specific communication sounds (Gerhardt, 1978; Liberman et al., 1958; Peters et al., 1980), and in neurophysiological studies of communication sound processing (Hall and Feng, 1986; Margoliash, 1983; Mueller and Leppelsack, 1985). Thus, it has been suggested that coincidence detection based on similar synaptic mechanisms may be used by the central auditory system to encode communication sounds (Olsen and Suga, 1991b; Suga, 1988). Whether or not this is true, however, remains to be tested.

Likewise, little is known about how the acoustic parameters that characterize communication sounds are represented in the central auditory system. For neural systems that encode sound source location, the acoustic parameters that carry spatial information are systematically represented by place within central neural structures to form maps of particular spatial dimensions of the external world (Wise and Irvine, 1985; Moiseff and Konishi, 1981; Olsen, Knudsen, and Esterly, 1989; Suga et al., 1983). The clearest examples of this are the maps of echo delay (target distance) in the auditory cortex of the bat, and the maps of interaural time difference (sound source azimuth) and interaural intensity difference (sound source elevation) in the inferior colliculus and optic tectum of the barn owl (Moiseff and Konishi, 1981; Olsen, Knudsen and Esterly, 1989). These maps are designed to represent, for each species, only the ranges of parameter values that are biologically important. However, no such mapping of the acoustic parameters of communication sounds has been found in any species. The lack of such maps in neural structures that represent communication sounds may be due to the fact that the species examined (eg., various songbirds and frogs; Fuzessery and Feng, 1983; Margoliash, 1983: Mueller and Leppelsack, 1985) show little natural variation in species-specific calls.

Most of what is known about the functional role of the MGN and auditory cortex in processing complex sound has come from studies of the mustached bat (Olsen and Suga, 1991a, b; Suga et al., 1983; Suga, 1990; Suga et al, 1990). In this species, the MGN and dorsal regions of auditory cortex contain combination-sensitive neurons that respond selectively to combinations of components from the the bat's biosonar pulse and echo. These combination-sensitive neurons are tuned to delays and small frequency differences between the pulse and echo, such as found between pulses and echoes emitted and heard by the bat during echolocation. The combination-sensitive neurons encode echo delay and Doppler-shift, cues used by the bat for determining the distance and relative velocity of a target. The MGN appears to be where this response property is created. In the MGN, combination-sensitive neurons are clustered in the dorsal and medial divisions. These divisions receive a convergent projection from tonopically organized regions of the inferior colliculus and project to the combination-sensitive areas of the auditory cortex. The neural mechanisms that produce the delay tuned neurons in the bat's MGN process acoustic delays in the range of 1 to a few tens of milliseconds, as needed for ranging in echolocation. Similar neural mechanisms may be used in other species for auditory tasks that require temporal processing within similar limits, such as the categorical perception of phonemes from voice onset time (Liberman et al., 1958). Thus, the data from the bat suggest that the MGN is not simply a relay from the inferior colliculus to the auditory cortex; rather, the MGN is a center for processing acoustic signals of biological importance. There are, however, few data from other mammalian species that address the role of the MGN and auditory cortex in processing complex sound. Most studies have been conducted in the cat and have used simple tonal timuli and/or unmodulated broadband noise. These studies have shown that the MGN has a perplexing tonotopic organization (Imig and Morel, 1985) and that a substantial proportion of MGN neurons do not respond to single tones (Calford, 1983). Recently, combination-sensitive neurons that are sensitive to sound spectrum have been discovered in a dorsal region of the cat's auditory cortex (Sutter and Schreiner, 1991). Whether or not similar neurons exist in the primate MGN or auditory cortex is not known.

Processing of species-specific calls by the central auditory system of the squirrel monkey

The squirrel monkey has a varied repetoire of calls. Different calls are are produced in different behavioral contexts. Most calls have been classified acoustically into several groups that differ from each other in harmonic structure, periodicity, and/or component type (Newman et al., 1983; Winter et al., 1966; Winter, 1969). Calls within a group share certain acoustic components, but vary in component frequency and duration, or in how components are assembled. Because different calls, whether acoustically related or not, differ in behavioral significance, it is likely that the auditory system of this species has evolved specialized auditory mechanisms to distinguish among them.

Previous studies of the MGN, auditory cortex (the superior temporal gyrus) and dorsolateral frontal cortex of the squirrel monkey have reported neurons that respond to token vocalizations, but respond poorly to "standard" auditory stimuli, such as tones, clicks and noise (Manley and Mueller-Preuß, 1978; Winter and Funkenstein, 1973; Newman and Lindsley, 1976; Newman and Wollberg, 1973a, b; Glass and Wollberg, 1979, 1983; Symmes et al, 1980; Allon et al, 1985). Although these results suggest that the stimulus requirements of these units is more complex than those of peripheral auditory neurons, they do not directly address the problem of response selectivity for vocalizations because, in most cases, the responses to vocalizations were compared to responses to acoustically dissimilar sounds, and because neural tuning to the acoustic parameters of calls were not completely measured.

In other studies, the responses of MGN neurons in the squirrel monkey to digitally filtered vocalizations were modeled by Volterra kernels (Yeshurun et al. 1985). This approach yielded the transfer function between the waveform of the input (ie., the token vocalization) and the waveform of the response output (ie., the poststimulus time histogram of the neuron's response). Although this approach provides a mathematical description of neural response patterns, it is not a test of call-selectivity and has not led to testable hypotheses about the neural mechanisms for call-selectivity.

Objectives:

The primary goal of this project is to determine the computational strategies and neural mechanisms for encoding and representing species specific-communication sounds in primates, about which little is known. For this, the squirrel monkey offers several advantages: Squirrel monkey calls are varied, yet highly stereotyped. They are classifiable into several groups of acoustically related calls. The behavioral significance of most calls is known (Winter, 1966; Winter et al., 1969; Newman et al., 1983). Although neurons that respond to squirrel monkey communication sounds have been found in the squirrel monkey's auditory cortex and medial geniculate body, the selectivity of these neurons for the monkey's communication sounds has not been rigorously tested. Moreover, for no species has a systematic representation of the acoustic parameters of communication sounds been demonstrated. I have been using neurophysiological and neuroanatomical techniques to study how the central auditory system of the squirrel monkey encodes and represents the monkey's calls. The MGN, rather than the auditory cortex, was selected for the initial studies for three reasons: First, there are the studies of the MGN of the mustached bat that indicate that the dorsal division of MGN is where combination-sensitive neurons are created. Second, the geometery of the MGN is is such that dorsoventrally oriented electrode penetrations through it are likely to pass through the dorsal division. In contrast, the auditory cortex is occupies a much wider area; thus, much time may be spent searching for combination-sensitive neurons in cortical areas that do not contain them. Third, once the thalamic regions that contain combination-sensitive neurons are identified, tracer injections of anterograde tracers into these regions will identify cortical areas for later physiological study. There are three specific aims of this study, embodied in the following questions:

1. Do the medial geniculate nucleus (MGN) and its cortical targets in the squirrel monkey contain neurons that respond selectively to combinations of call components and that are tuned to the acoustic parameters that characterize naturally occurring calls, analagous to the combination-sensitive neurons found in the central auditory system of bats and lower vertebrates? I am seeking combination-sensitive neurons that are tuned to the acoustic parameters that characterize individual components of squirrel monkey calls (eg., frequency, amplitude, duration, degree and rate of frequency modulation) and to particular parameters defined by combinations of components (eg., frequency ratios and time intervals). The values of these acoustic parameters to which these neurons are tuned are expected to fall within the ranges of those that characterize naturally occurring vocalizations.

2. What are the neural mechanisms by which call-sensitive neurons are created? Neurons that are tuned to call spectrum are expected to arise though a convergence within a tonotopically organized system, as shown for combination-sensitive neurons that process biosonar information in the bat (Olsen and Suga, 1991b; Suga, 1990). For no species, however, have the neural mechanisms that process communication sounds been revealed. Neurons that are tuned to time intervals between call components are expected to be sensitive to the coincidence of activity evoked by separate acoustic events, as found for delay-sensitive neurons in other species (Rose et al., 1966; Sullivan and Konishi, 1986; Olsen and Suga, 1991b; Suga, 1990).

3. Are the acoustic parameters that characterize squirrel monkey's calls mapped in central neural structures? I am seeking evidence for a functional organization of neurons according to their tuning to particular acoustic parameters, eg., rate of frequency modulation, and frequency ratios or time intervals among signal elements. No such mapping has been found in systems that process communication sounds. I expect to find that call-selective neurons are clustered in specific areas of auditory cortex and in the dorsal and medial divisions of the MGN, as is the case for combination-sensitive neurons in the mustached bat (Suga, 1983; Olsen and Suga 1991a, b). I will determine if these areas have anatomical axes for the representation of acoustic parameters that characterize or distinguish among particular communication sounds.

Methods:

Acoustic analysis of squirrel monkey calls

Samples of squirrel monkey calls are recorded and digitized on a 486 type computer. Spectrographic analysis (RTS™ by Engineering Design) is used to determine the fundamental and harmonics of the call. The fundamental is divided into components based on direction, depth and rate of frequency modulation. Then, the acoustic parameters that characterize and interrelate call components are measured from their spectrographs. Average values are determined for individuals and for the population. Crosscorrelations of spectrograms are performed to determine the acoustic similarities among components from different types of calls. This analysis leads to identification of components shared among different calls and identifies the temporal and spectral differences among them. The information obtained from the acoustic analysis of the monkey's calls is used to create computer models of call components which are then used as auditory stimuli in neurophysiological recording, as described below.

Auditory stimuli

The stimuli used in this study consist of tones, synthetic calls, natural calls, and components of synthetic and natural calls. Auditory stimuli are presented under free field conditions to halothane-anesthetized monkeys in a sound-isolation chamber.

Tones are used to measure frequency and amplitude tuning of auditory neurons. For this, a tone burst, 100 ms in duration is generated, gated to remove transients, and presented at frequencies from 20-20,000 Hz and amplitudes from 0-100 dB SPL. Synthetic calls and their components are used to measure neural tuning to acoustic parameters of individual components (such as component frequency, amplitude, duration, degree and rate of frequency modulation), and tuning to parameters defined by pairs of components (frequency ratios and time intervals). Call components are modeled by 2 functions: the first describes call frequency versus time, whereas the second describes and call amplitude versus time. The principal advantage to using models (as opposed to using edited natural calls) is that the models can be rapidly and systematically modified in the frequency, amplitude and time domains. With the models, call components are frequency shifted, amplitude modulated, rearranged, expanded, and compressed by arithmetic operations on the models themselves. Calls and their components are resynthesized from models with signal processing software (Signal™ by Engineering Design) that emulates a voltage controlled oscillator. Amplitude modulation is then introduced by multiplying the resulting time signal with the model envelope.

Several months have been invested in customizing the modeling software so that model calls can be modified interactively while recording responses of auditory neurons. With the program virtually all call parameters may be independently varied: component frequency and duration, rate of frequency modulation (FM), depth of FM, direction of FM, duration of FM. In addition, permutations of two or more components can be generated rapidly and intercomponent intervals can be varied systematically. As described below, intercomponent interval was found to be critical to the responses of some MGN neurons.

Recording from MGN neurons

Action potentials from neurons in the MGN are recorded extracellularly with tungsten wire microelectrodes. The signal from the electrode is amplified, filtered and input to a window discriminator (BAK Electronics) to isolate individual units. Unit activity is counted by a 486 type computer running data collection software (Spike-2™ by Cambridge Electronic Design). The software stores spike arrival times (re. the stimulus) and constructs raster displays and peristimulus-time histograms on line.

Major Findings:

Acoustic analysis of SM calls

Thus far, the acoustic analysis of squirrel monkey calls supports their division into two classes: tonal calls (eg., peep, chuck, yap, twitter, cackle) and noisy calls (eg., err, shriek, girren), in agreement with previous studies (Newman et al., 1983; Winter et al., 1966; Winter, 1969). The components of the tonal calls were classified as long upward-sweeping FM, long downward-sweeping FM, short up FM, short downward FM, long constant frequency (CF), and short CF. The analysis revealed strong acoustic similarities between the chuck, peep, yap and cackle calls. Of these tonal calls, the chuck was particularly interesting because it has at least one component in common with the other 3 calls. The chuck is a stereotyped and broadband call (bandwidth > 19 kHz) that consists of an long upward FM component, followed immediately by a short downward FM component and a short CF component. These three components were labeled C1, C2, and C3, respectively. Considered individually, the 3 components of the chuck are acoustically similar to vocalizations produced in other contexts; specifically, C1, C2 and C3 of the chuck are individually indistinguishable from peep, yap and cackle calls, respectively. Thus, the 4 calls (chuck, peep, yap, and cackle) represent an auditory discrimination problem for the monkey and a related computational problem for the central auditory system. For this reason, the chuck was modeled and used extensively in the initial neurophysiological experiments.

Responses of MGN neurons to chuck calls

The chuck was modeled on a computer and the 3 constituent components (C1, C2 and C3) were presented singly and in combination to a halothane-anesthetized monkey while responses of MGB neurons were recorded. Thirty neurons were found that responded reliably to the model chuck presented at 70 dB SPL; of these, 8 responded only poorly to the three components presented individually. When two or more components were combined as found in the natural chuck, these 8 neurons were facilitated; gating the components individually, or introducing more than 5 ms of delay between them abolished the facilitated responses. In addition, 5 other neurons were found that responded well to the third component presented alone and poorly to the third component preceded by the second component. For these C3-responding neurons, the responses to C2-C3 pairs remained suppressed (ie., less than the responses to C3 alone), for C3 amplitudes up to 24 dB louder than C2. Reconstruction of recording sites (one animal) revealed that these specialized responses were located in dorsal and medial regions of the MGN. Thus, the data indicate that certain neurons in the monkey's MGN are facilitated by combinations of call components, whereas others are selective for individual components. The discovery of combination-sensitive neurons in the MGN of the monkey is the critical first step in understanding the neural mechanisms by which communication sounds are processed by the primate central auditory system.

Proposed Course of the Project:

The results of the pilot phase of this study have been highly encouraging. The following experiments are planned to further address the three specific aims.

1. Call-selectivity of combination-sensitive neurons in the MGN

Additional neurophysiological recording experiments are needed to determine the degree to which thalamic combination-sensitive neurons are selective for particular vocalizations. With the computer-generated models of calls and call components, I will measure the tuning of combination-sensitive neurons to the acoustic parameters that characterize individual components of squirrel monkey calls (component frequency, amplitude, duration, degree and rate of frequency modulation) and to particular parameters defined by combinations of components (ie., frequency ratios and time intervals between components). To measure tuning to a particular acoustic parameter, a series of model calls is generated for which the selected parameter is varied systematically. The series of model calls is presented to the monkey while the responses of MGN neurons are recorded extracellularly, as described above. The parameter value evoking the largest response may then be determined. Call selectivity will be assessed by comparing the acoustic parameters to which these neurons are tuned with the parameter ranges that characterize naturally occurring vocalizations.

2. Neural mechanisms of combination-sensitivity

Once the tuning of call-selective neurons in the frequency, amplitude and time domains is known, I will test the probable neural mechanisms that produce the specialized filter properties of these neurons. Given the sensitivity of some MGN neurons to time intervals between call components, I will next measure latencies of responses to individual components to determine if the delay sensitivity is based on a sensitivity to the coincidence of excitation evoked by individual components. Temporal patterns of response, as revealed in peristimulus time histograms, will be examined to determine the time course of inhibition and facilitation.

If neural tuning to call spectrum or frequency ratio is found, then the anatomical basis for this sensitivity will be sought, as described below. Extracellular deposits of horseradish peroxidase and other axonally transported anatomical tracers will made to identify the afferent and efferent connections of thalamic regions that contain combination-sensitive neurons. I will be seeking evidence for a convergence within a tonotopically organized system that may explain the spectral selectivity of combination-sensitive neurons. The anatomical data will be evaluated in terms of what is known about tonotopic organization of structures that provide input to area of interest. The anatomical studies will, in addition, identify the cortical targets of thalamic regions that contain combination-sensitive neurons. These cortical regions will then be examined physiologically for evidence of combination-sensitivity, as described above.

3. Functional organization of combination-sensitive neurons

Physiological mapping techniques will used to determine the distribution of combination-sensitive neurons in the MGN and in the identified cortical targets of the MGN. I will be seeking evidence for a functional organization of neurons according to their tuning to particular acoustic parameters. I will determine if these regions have anatomical axes for the representation of acoustic parameters that characterize these communication sounds, eg., frequency ratio or time interval among signal elements. These experiments will involve stimulation with model calls and extracellular recording of neural responses, as described above. Recording sites will be marked with small electrolytic lesions and electrode tracts will be reconstructed with the aid of immunocytochemical staining for glial fibrillary acidic protein (Benevento and McCleary, 1992).

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

The discovery of combination-sensitivity in the monkey's MGN, similar to that described in lower vertebrates, suggests a general role this neural response property in the processing of information in complex, biologically significant sounds. The sensitivity of these neurons to time delays between signal components has a clear parallel with the categorical perception by humans of phonemes based on voice

onset time. Thus, the results suggest that, in primates, combination-sensitivity and delay-sensitivity may reflect neural computations for categorizing acoustically related vocalizations that differ in their behavioral significance. Future research will focus on determining the neural mechanisms by which combination-sensitivity and delay sensitivity are created and on determining the representation of communication sounds in higher auditory structures. Evidence will be sought for transmission delays and for convergence in the afferent pathways to the MGN. The MGN and its cortical targets will be examined for maps of the acoustic parameters of communication sounds. I expect that this line of inquiry will lead to a greater understanding of the neural mechanisms of language processing and may lead to animal models to study the pathology of Wernicke's aphasia and other language disorders.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER:

ZO1 MH 01103-01 LNP

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Role of the Frontal Lobe in Control of Memory-Guided Behavior

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

PI: Roger G. Erickson Senior Staff Fellow LNP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Animal Center, Poolesville, Maryland 20837

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

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

I have initiated a study of the behavioral neurophysiology of the prefrontal cortex. A new laboratory for studying eye movements, single-neuron and behavioral responses was set up in order to evaluate the relation between neuronal activity in the prefrontal cortex and conscious control of visually-triggered delayed response tasks. A computer-driven video projector system has been developed to allow stimulation of larger portions of the visual field and a new program has been installed to allow more efficient and flexible control of behavioral routines. Several monkeys were trained on novel variations of task requiring memory-guided eye movements and experiments were initiated to determine whether the activity of prefrontal neurons correlates with distinct stages or strategies necessary for solution of these tasks.

Neuronal recordings are now underway and will continue throughout the coming year.

Objectives:

I intend to provide an explanation of how prefrontal neuronal activity relates to the process of integrating memory-guided and sensory-guided motor processes. It has been proposed that a major role of the prefrontal cortex is to contribute to short-term memory storage. To accommodate current data, however, it is necessary to expand this hypothesis to consider how monkeys regulate use of the alternate strategies of basing behavior upon stored data vs current exteroceptive information. While it is clear that some prefrontal neurons contribute to the solution of delayed-response tasks, their role has not been clarified. It is not even known whether they contribute to the storage or retrieval of enteroceptive data, to the formulation of motor responses, or rather to the selection of the appropriate strategy. To further define that role, several variations of the delayed-response task have been implemented in order to separately examine its successive stages.

Methods:

The project is carried out entirely with rhesus monkeys, using approximately two monkeys per year. The procedures include (1) chronic skull implants for head immobilization and electrode access, and implantation of scleral search coils for precise measurement of eye movements, (2) daily mounting of a small microelectrode drive and insertion of electrodes, (3) daily training or experimental sessions for liquid rewards, (4) computerised real-time control of behavioral tasks and data acquisition, and (5) perfusion and histochemical analysis of brain tissues after conclusion of the experiment.

(1) Surgical procedures to attach chronic implants is performed using deep pentobarbital anesthesia and sterile technique as directed by the Facility Veterinarian. A headholder consisting of a hollow stainless steel pedestal is attached by deflecting the scalp and anchoring directly to the skull with finely threaded stainless steel screws and dental acrylic. Eye coils are attached at the same time by blunt dissection to enlarge a tiny incision in the conjunctival connective tissue surrounding the outer part of the eye. The sterile, Teflon coated stainless steel coil is simply inserted into this pouch encircling the eye before the pouch is drawn closed with two sutures placed at the medial and lateral edges of the eye. Leads from each eye are then passed beneath the skin to a small connector embedded in the acrylic forming part of the pedestal already on the top of the skull.

The cylinder allowing chronic neuronal recording is implanted last, after the animal is fully trained, using the same technique as used for the headholder, with the exception that the flap of bone directly beneath the cylinder is opened with a trephine and removed to allow electrodes to approach the exposed dura.

(2) The recording cylinder is normally sealed with a sterile plastic cap that is cleaned and flushed with sterile saline, and infused with ophthalmic antibiotic daily during experiments, or at two day intervals during vacations from experiments.

(3) The behavioral tasks involved require the monkey to be restrained in a standard primate chair for 3-4 hrs daily, during which time the animal works to satiety for its daily liquid intake. Each animal is weighed at least weekly during training or experimental periods and is given water ad lib for at least one day each week to ensure adequate

hydration. The intent of the behavioral protocol is to isolate and examine each of the major stages involved in solution of the task. The stages of interest are as follows, (a) visuospatial memory storage, (b) detection of alternative visuo-sensory information, (c) instructed choice of behavioral strategy, (d) formulation of oculomotor plan, (e) execution of memory guided or sensory guided oculomotor saccade, (f) memory guided or sensory guided fixation, and finally, the reward.

The task events are initiated when the monkey presses a bar to illuminate a central, white fixation point (FP), which they must thereafter fixate attentively. After a short delay an eccentrically placed visual cue (memCue) is briefly illuminated and extinguished. After a variable delay a second visual cue (visCue) is illuminated and left on at a different location. After further delay the color of the white FP is changed to indicate which stimulus the monkey is to respond to. If the FP remains white the monkey's task is to ignore the cues and simply wait for a brief pulse in the luminance of the FP before releasing the bar for a liquid reward. If the FP changes color the monkeys task is to wait until the FP goes out before making a saccadic eye movement to the location of one of the two cues. A red FP color requires a saccade to the remembered location of the first cue (memCue), and a green FP color requires a saccade to the visible cue (visCue). After a saccade, the monkey must withold bar release and scrutinize the intended location until luminance of the darkened or visible cue is also pulsed. Past results have already indicated that periods of altered activity which are evoked in profrontal neurons during the course of this task will be correlated with one or more of the events signalling the above task stages.

Two monkeys have now been trained on this task. The training schedule was delayed substantially when, after more than three months of initial training, the first monkey was found to have a congenital abnormality of fixation which precluded his further use in the study. Training of the second monkey was then completed and recordings are proceeding in this animal. A third monkey is approximately 2/3 through the training process and will be implanted with a recording cylinder as soon as remodeling of our surgery suite is completed (sometime within the next two months).

(4) Training and experimental sessions are now controlled by a new software package, custom designed for this lab, which allows on-line development and alteration of behavioral protocols, stimulus presentation and data collection. This software package has just been ported to a more powerful, pc-based platform and is currently being installed in the lab. It is expected that the new software will significantly decrease the time required to develop new protocols and train animals since it allows user-set or automatically incremented on-line adjustment of protocol variables without interrupting ongoing trials. The program should also significantly enhance the rate of data collection since it also allows stimulus parameters to be easily and immediately adusted on-line to match the optimum requirements of each neuron, rather than searching for neurons that can be driven with a fixed set of stimulus variables

(5) After conclusion of experiments in a given animal, the body is perfused with buffered saline followed by a buffered aldehyde solution to fix the CNS and the brain is blocked and sectioned on a freezing microtome to allow reconstruction of electrode tracks and recording sites.

Major Findings:

Monkeys are readily able to solve more difficult versions of the delayed-saccade tasks and can also be trained to perform several different tasks in a randomly interleaved sequence. Previous work by others using delayed-saccade tasks has shown that many neurons anterior to the frontal eye fields are tonically active during the delay period while a monkey is waiting to make a memory-guided saccade to particular locations. My preliminary work has shown that the activity of such neurons ends upon completion of the saccade and that other prefrontal neurons become tonically active only when a monkey tries to maintain a stable memory-guided gaze position in total darkness. These data suggest that prefrontal neurons may not contribute directly to memory storage or motor planning, but that activity reminiscent of these processes is seen whenever a monkey is using stored information to control it's behavior. This view is consistent with the hypothesis that information from many brain regions is available to the prefrontal cortex, but that such information is brought on-line only when it is used to guide ongoing behavior.

Progress made during the past year has been to complete construction and set up of the laboratory equipment, to train monkeys on the behavioral tasks, to consult on the final design and install a new laboratory software package for training and data collection, and to start neurophysiological recordings.

Significance To Biomedical Research and to the Program of the Institute

The purpose of this project is to improve understanding of the role of the prefrontal cortex in coordinating the ability to organize behavior alternately upon memorized or current sensory information. It has been known for over 100 years that damage to the frontal lobe is associated with an increased tendency to make frequent and non-purposive movements. In recent years there has also been renewed interest in the many observations that frontal lobe damage is associated with frequent compensatory saccadic intrusions when attempting smooth pursuit and with deficits on delayed response tasks. The observation that focal prefrontal lesions may selectively impair the ability to generate appropriate memory-guided limb movements while sparing other memory-based motor acts such as speech suggests a modular organization of elements in the prefrontal cortex which allow specific types of motor planning to be based upon stored as opposed to current sensory data.

Proposed Course of the Project:

I plan to use the methods and tasks developed this year to examine three related hypotheses.

(a) Correlation of prefrontal neuronal activity with short-term memory storage. It has been proposed that delay-period activity observed in the prefrontal cortex during delayed-saccade tasks is a representation of short-term or 'representational' memory. My variations of the task will allow me to determine whether the onset of such activity

correlates with information storage or with the decision to utilize that information to prepare a motor response.

(b) Correlation of posterior parietal neuronal activity with short-term memory storage and motor planning. Neurons in parietal area LIP as well as within the superior colliculus are also active during delayed-saccade tasks. While collicular neurons may be a link between cortical areas and the oculomotor nuclei, it is assumed that parietal and prefrontal areas make different contributions to successful execution of a delayed oculomotor response. Therefore, comparison of parietal and prefrontal responses on the tasks I have prepared would be very useful in assessing possible differences in their contributions. My initial expectation is that parietal areas provide the spatial information necessary to program a memory-guided eye movement, while the prefrontal cortex acts as the conditional interface which allows motor areas to be activated by stored rather than direct sensory information. If true, parietal neurons would be activated upon memory storage in my tasks, while prefrontal neurons would become active only with the decision to use the stored info to prepare a motor response.

(c) Spatial vs retinotopic organization of memory for cue location in delayed-saccade tasks. Changing the location of the FP after presentation of a peripheral cue will allow me to see if monkeys can make memory-guided saccades to spatiotopically as opposed to retinotopically specified coordinates. Undoubtedly they can do both, but it is not at all clear how such a subtle change in strategy would be reflected in the response profile of prefrontal or parietal neurons.

The present experiments all deal with the role of the prefrontal cortex in coordinating spatially organized behavior. In future years this project will be elaborated in two directions. One branch will investigate the neural mechanisms by which monkeys, when given a clear choice, make and initiate execution of their decision to base behavior upon memory-based as opposed to sensory-based information. The other branch will investigate the role of the prefrontal cortex in an animals ability to make temporal predictions and execute temporally patterned motor responses.

Publications:

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01104-01 LNP

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Neurobiology of Attachment and Separation

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

PI:	T. R. Insel	Staff Physician	LNP, NIMH
	D. M. Witt	NRC Fellow	LNP, NIMH
	J. T. Winslow	Staff Fellow	LNP, NIMH

COOPERATING UNITS *(if any)*

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Department of Zoology, University of Texas, Austin, TX
Department of Endocrinology, Univ. of Pittsburgh School of Med, Pittsburgh, PA

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Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Animal Center, Poolesville, Maryland 20837

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

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

This project represents the integration of two former projects from the defunct Section on Comparative Studies in the Laboratory of Clinical Science (The Neurobiology of Attachment, Z01 MH 00797-LCS and Animal Models of Anxiety Z01 MH 02219-LCS). The central focus of this new project is the neural basis of affiliative behaviors; specifically the role of the neuropeptide oxytocin in several aspects of affiliation. Behavioral studies in the rat, which originally focused on maternal behavior, have been expanded to explore oxytocin's role in sexual behavior, social interaction, and the infant's response to separation. Cellular studies of the mechanisms of oxytocin's effects have continued to focus on the regulation of oxytocin receptor expression. These receptors in brain appear to be highly plastic. In the rat, expression of oxytocin receptors in specific brain regions is almost entirely dependent on gonadal steroids. By contrast, in selected regions of the mouse brain, oxytocin receptors may be inhibited by gonadal steroid stimulation. The oxytocin receptor has also emerged as an important factor in comparative studies of microtine rodents (voles) with contrasting patterns of affiliation. Last year we reported that the regional distribution of oxytocin receptors differed markedly between monogamous and polygamous voles. This year, we have explored the functional significance of these species differences in receptor distribution. In the monogamous prairie vole, oxytocin administered intracerebroventricularly induces a partner preference in the female and an increase in aggression in the male, effects which are critical to the initiation of a pair bond. Paternal behavior in this species releases oxytocin and is selectively decreased by amygdala lesions. In the polygamous montane vole, oxytocin has little influence on several measures of social behavior. The accumulated behavioral, receptor, and neuroanatomic evidence suggests that central oxytocin projections may have evolved in mammals to mediate diverse forms of attachment behavior.

Other Collaborative Professional Personnel Engaged in the Project:

Brian Kirkpatrick	Asst. Prof.	Dept. Psychiatry	MD Psychiatric Research Ctr.
C. Sue Carter	Prof.	Dept. Zoology	University of Maryland

Project Description:

Objectives: Over the past six years we have focused on the neural mechanisms of social attachment. Our behavioral studies have implicated oxytocin as a neuropeptide uniquely important for attachment behavior. The major goal of this project has been to understand how this neuropeptide system can mediate such a diverse range of social behaviors.

Methods Employed: Behavioral studies in adult rats have assessed reproductive behaviors, social interaction, parental care, and infant separation responses in rats. Reproductive behavior in the female includes not only lordosis but a number of proceptive and receptive responses that are evaluated by a trained rater using computer assisted observational monitoring. Social interaction is studied for prolonged intervals using time-lapse video technology. Parental behaviors are assessed in the home cage and provide scores of offspring retrieval and contact. In rat pups, separation responses are studied within the first 5 minutes of social isolation. Our major dependent measure for the rat pup is the ultrasonic isolation call. During this year, we changed our computer-based system for detecting calls to allow long term storage of sonographic records of ultrasounds. In addition, we collect several concurrent measures from pups: locomotor activity, rectal temperature, and geotaxis.

Increasingly, our behavioral studies have shifted from rats to a species with long-term, selective social bonds- the prairie vole. Voles are housed in a burrow system which simulates field conditions. Social preference studies are accomplished in a 3 chamber choice paradigm with exposure to tethered social partners. Paternal behavior studies use a similar multi-chamber choice paradigm. All behavior is recorded with time-lapse videotape for subsequent scoring by a rater blind to treatment condition.

Peptides are administered either intracerebroventricularly (icv) or locally by a chronic cannula implanted stereotaxically. As the thin skull of the vole prohibits chronic cannula placement, we have developed a method for icv administration using subcutaneous osmotic minipumps attached to an intracranial lateral ventricular port. Axon-sparing lesions are made with stereotaxic injection of N-methyl-d,l-aspartate.

Oxytocin receptors are studied quantitatively using *in vitro* receptor autoradiography with ^{125}I-OTA--a high affinity oxytocin antagonist. This analogue has a Kd for oxytocin receptors in the 50-100 pM range, 50-100 times more potent than its affinity for vasopressin receptors. ^{3}H-oxytocin, the ligand used previously, binds to oxytocin receptors with only slightly higher affinity than it binds to vasopressin receptors--thus complicating the interpretation of results. For comparison to oxytocin receptor maps, alternate sections are studied for vasopressin receptor distribution using a new iodinated vasopressin analogue, ^{125}I-Sarc-AVP.

Classical immunocytochemistry with the PAP-DAB method has been used for localizing cells expressing oxytocin, vasopressin, and the protein product of the c-*fos* oncogene. The expression of c-*fos* provides maps of activated circuits following seizures, dehydration, or drug administration in brain. We have validated our technique by replicating earlier results of increased c-*fos* expression in dentate gyrus following pentylenetetrazol-induced seizures. Double labelling for c-*fos* protein and oxytocin and vasopressin is accomplished with both monoclonal and polyclonal antibodies for the neuropeptides.

Expression of oxytocin and vasopressin mRNA in rat and vole is accomplished with both *in situ* hybridization histochemistry and Northern analysis. The former uses a series of different oligonucleotide anti-sense probes (voles different than rats) that are 3'-end-labelled with ^{35}S and analyzed by both film autoradiography and darkfield microscopy of emulsion-dipped sections. Northern analysis uses ^{32}P-labelled riboprobes transcribed from cDNAs subclones of exon C of the vasopressin and oxytocin genes.

Major Findings:

1. Oxytocin's central effects on behavior have been shown to include affiliative behavior as well as maternal and reproductive behavior. In rats, the physiologic role of oxytocin in female reproductive behavior has been demonstrated with both intracerebroventricular and site-specific injections of an oxytocin antagonist. A similar role now sems likely in males as ejaculation is associated with c-fos protein induction in oxytocin cells in the paraventricular nucleus of the hypothalamus.

2. We have previously shown that oxytocin receptors are more abundant in the infant than in the adult rat brain. We have now found that oxytocin decreases rat pup ultrasonic vocalizations when given centrally (but not when given peripherally). Equivalent doses do not change several concurrent measures of arousal or thermoregulation. Oxytocin effects are blocked by the oxytocin receptor antagonist OTA, but OTA has no effects alone when given to isolated pups or pups exposed to a social companion. Vasopressin also decreases rat pup ultrasonic vocalizations in a dose-dependent fashion. These effects are not mediated by the oxytocin receptor, but are blocked by an equimolar dose of a V_1 antagonist.

3. Oxytocin's behavioral effects are mediated by membrane-bound receptors. In the rat brain, these receptors show remarkable plasticity. In specific regions (bed nucleus of the stria terminalis and ventromedial nucleus of the hypothalamus), these receptors increase 100-300% in response to gonadal steroids. Physiologic changes in gonadal steroids (e.g. at puberty, parturition, and estrus) are associated with increases in both local oxytocin receptor concentration and functional responses to oxytocin. Decreases in oxytocin following lesions of the paraventricular nucleus of the hypothalamus do not alter oxytocin receptor number or distribution, although chronic central infusions of oxytocin can decrease receptors by as much as 90%.

4. The plasticity of brain oxytocin receptors is also apparent in the differences in their distribution across species. Maps of forebrain oxytocin receptors across 9 different rodents show few overlaps. Species differ not only in the distribution of receptors, but in the regulation of receptor expression by gonadal steroids. In the mouse, gonadal steroids decrease rather than increase oxytocin receptors in the ventromedial nucleus of the hypothalamus.

5. Voles with different patterns of social organization have different patterns of oxytocin and vasopressin receptor distribution. These patterns are associated with different behavioral responses to exogenous peptides. In the monogamous prairie vole, intensive bouts of mating are followed by the development of a partner preference in the female and an increase in aggression towards novel conspecifics in the male. These rapid behavioral changes appear to be fundamental to the formation of the long-term selective social bond in this species. Central (but not peripheral) administration of oxytocin induces a partner preference in the female even in the absence of mating. In the male, central administration of vasopressin increases aggression, a selective vasopressin antagonist blocks the mating-induced aggression, and mating is associated with an increase in vasopressin mRNA. In the polygamous montane vole with a different pattern of brain receptors for oxytocin and vasopressin, these peptides have relatively weak effects on social behaviors.

6. The male prairie vole is an excellent animal for the study of paternal behavior. Excitotoxic lesions of the medial nucleus of the amygdala decrease paternal care of pups without affecting several other forms of social or non-social behavior. Lesions of the same region in females do not reduce maternal behavior.

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" has 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. Deficits in social interaction are characteristic of autism, schizophrenia, and certain forms of character pathology.

Data from our group increasingly point to the brain oxytocin system as an important component of the neural mediation of diverse affiliative behaviors including parental care, sexual behavior, social grooming, and pair bond formation. The discovery that gonadal steroids alter the expression of both the peptide and its receptors suggests that some of the behavioral effects ascribed to estrogen and testosterone may be mediated by changes in oxytocin neurotransmission. We believe that this relationship will prove to be an excellent example of a general rule--namely, that steroids in the brain regulate the genomic expression of peptides and their receptors. The elucidation of this neurobiological relationship and the description of its consequences should provide insights into a number of clinical psycho-neuro-endocrinopathies including the mental symptoms associated with postpartum and pre-menstrual states as well as abnormalities of parental care (e.g. child abuse and neglect).

Proposed Course: The prairie vole continues to provide an ideal model for this project because the process of pair-bond formation occurs rapidly (less than 24 hours of socio-sexual interaction) and is associated with profound behavioral changes (increased aggression in the male and partner preference formation in the female). The neurobiological changes that subserve these behaviors will remain a major focus in the coming year. In the coming months, we will investigate both the release (radioimmunoassay) and the synthesis (mRNA) of oxytocin and vasopressin in response to social experience in the prairie and montane vole. Ongoing studies use selective antagonists for oxytocin and vasopressin to determine how central blockade of these systems alters the behaviors associated with pair bonding in the prairie vole. The recent cloning of the human uterine oxytocin receptor is of vital importance for this project. This published cDNA will provide a template for the development of both probes for the brain receptor mRNA (to study receptor synthesis) and antibodies to investigate the localization of receptors at the cellular level. Using molecular approaches, we should finally be able to determine the extent to which species differences in receptor distribution reflect differences in receptor structure. As a longterm goal, we are interested in developmental influences on adult social behaviors. Earlier cross-fostering studies showed little effect of postnatal environment on adult species-typical patterns of affiliation. The use of embryo transfer technology should provide a tool for examining the prenatal environment as a determinant of adult behaviors.

Publications:

Journal Articles

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Book Chapters

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Insel TR. Long-term neural consequences of stress during development: Is early experience a form of chemical imprinting? In: Carroll BJ, ed. The Brain and Psychopathology. New York: Raven Press, 1991;133-152.

Insel TR. Oxytocin and the neuroendocrine basis of affiliation. In: Shulkin J, ed. Hormones, Mind, and Brain. Academic Press, in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBERZ

ZO1 MH 01105-01 LNP

PERIOD COVERED

October 1, 1991 to September 30, 1992

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 Research Biologist LNP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Animal Center, Poolesville, Maryland 20837

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

(a1) Minors

(a2) Interviews

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

This report covers the continuation of work which has in previous reporting periods been carried out within the Laboratory of Clinical Science, NIMH (ZO1 MH 00798 LCS and ZO1 MH 00799 LCS) and has now been consolidated into a single project. This work focuses on the normal development of the cerebral cortex in mice and rats using neuroanatomical techniques to study developing axonal connections. Our efforts have concentrated on the corticospinal and the callosal projections and have emphasized the role played by collateral elimination in the development of cortical connections.

Experiments using anterograde tracers to examine the disposition of the corticospinal axons have shown that these attain a normal configuration in dysmylinated jimpy mutant mice. This argues against the notion that early myelin may determine the trajectory of the developing corticospinal projection. Experiments using retrograde tracers to label corticospinal neurons or callosally projecting neurons in jimpy mice have shown that the tangential distributions of these two projection systems undergo the normal developmental restriction in these mutant mice. This indicates that maturation of function accompanying myelination is not critical to the normally occurring developmental collateral elimination within these cortical projection systems.

Experiments using heterotopic cortical transplants have identified position within the tangential plane of the cortex as a critical factor in determining which of the initially extended projections, cortical neurons maintain. Results of these and other studies provide support for the idea that similar cortical cell types are initially present throughout the tangential plane of the neocortex and that regional differences in mature cortical projection patterns may not be prespecified. However, our recent behavioral observations indicate that while homotopic cortical transplants can partially ameliorate some behavioral deficits that follow neonatal cortical lesions, heterotopic transplants do not, despite their seemingly appropriate projections. Thus, although position within the cortex influences the projections cortical neurons are able to maintain, early cortical neurons may indeed be, at least in some respects, prespecified as to their cortical region.

Other Collaborative Professional Personnel Engaged on the Project:

T.M. Barth, Assistant Professor, Texas Christian University, Fort Worth, TX

Objectives:

The overall goal of this project is to gain a better understanding of the development of the brain and, especially, of the cerebral cortex. We have concentrated our studies on the role certain regressive or eliminatory phenomena play during normal cortical development. It is now clear that these are critical in shaping the projection patterns that are found in the adult cortex. We have also examined a potential role played by glia in the development of one of the major cortical projections, the corticospinal tract.

Methods Employed:

The experiments completed or in progress can be considered in three separate groups:

1.) Considerable attention has been given recently to the neurite growth inhibiting properties of central myelin. It has been suggested that central myelin's strong inhibitory effects on growing neurites may be partly responsible for the absence of any substantial axonal regeneration following injury to the mature brain or spinal cord and that the myelination of early formed pathways could act as a negative influence affecting the trajectory of later arriving axons. In particular, it has been suggested that the neurite inhibitory properties of myelin present in the early developing ascending sensory bundles in the dorsal funiculus of the spinal cord serves a negative guidance function, "channeling" the late arriving corticospinal axons and confining their distribution to a bundle wedged in the ventral most portion of the dorsal funiculus. If this were true, then the absence of myelin in the ascending sensory bundles of the spinal dorsal funiculus should allow the developing corticospinal axons to disperse more widely, perhaps even spreading throughout the dorsal funiculus. To examine the disposition of corticospinal axons which have developed in a dysmyelinated spinal cord I utilized anterograde axonal tracing techniques in jimpy mutant mice. Jimpy is a recessive X-linked, juvenile-lethal mutation in which the lack of oligodendrocyte differentiation, together with frank oligodendrocyte loss, results in the virtual absence of myelin from the CNS.

2.) The developmental restriction of the tangential distributions of cortical projection neurons through collateral elimination occurs fairly late in cortical development and, in at least some systems, at around the same time as the relevant axons become myelinated. Observations from several studies suggest that neuronal activity may be involved in the determination of which collaterals are eliminated and which are maintained. Since myelination clearly contributes to the functional maturation of developing axonal systems, the possibility that myelination may be causally related to collateral elimination would seem to merit serious consideration. To examine the distributions of neocortical commissural and corticospinal neurons which develop in the virtual absence of central myeline I used retrograde tracing techniques in dysmyelinated jimpy mice, and in unaffected littermate controls, during the first and third postnatal weeks.

3.) During early development many cortical projection neuron populations are distributed widely and continuously across the neocortex. The redistribution of the projection neuron populations which results in the adult discontinuous pattern is due to selective collateral elimination with neurons in each cortical region maintaining projections appropriate to the function of that cortical region and losing the inappropriate collaterals which they initially had extended. This mode of cortical development 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 during development, since cortical projection neurons initially project to several targets, but only maintain collaterals that are appropriate for their locale. Consistent with this idea is our subsequent finding that neurons within pieces of fetal cortex transplanted heterotopically always maintain projections appropriate to their new cortical locale rather than to their region of origin.

If heterotopic fetal cortical transplants make and maintain projections appropriate to their new cortical locale, is placing a heterotopic transplant into a neonatal cortical lesion in all ways equivalent to the placing of a homotopic transplant? We have begun to explore this question by making lesions in the rostral cortex of newborn rats and transplanting pieces of either rostral or occipital fetal cortex into the lesion site (additional control animals are lesioned, but receive no transplant). When the animals mature, behavioral testing is done to identify residual deficits in the lesioned animals, to determine if the fetal transplants can at all ameliorate these deficits, and if so, to identify any difference in the effectiveness of the rostral and occipital transplants. In addition, the viability of the transplant is examined anatomically.

Major Findings:

The major findings of these three groups of studies can be summarized as follows:

1.) Corticospinal axons in jimpy mice follow a completely normal trajectory throughout their course, and form just as cohesive a bundle within the dorsal funiculus of the spinal cord as that found in mice with a normal complement of CNS myelin. Myelin within the sensory fasciculi of the dorsal funiculus is thus unlikely to play a critical role in pathway selection during the development of the corticospinal projection and does not serve to confine the corticospinal fibers to a compact bundle.

2.) In 16 to 19 day old mice the distributions of both callosally projecting neurons and corticospinal neurons have become discontinuous. The overall cortical distributions of these cortical projection neurons in jimpy mice are indistinguishable from those seen in the cortex of the control mice. Thus the congenital absence of CNS myelin in jimpy does not affect the normal developmental sculpting through collateral elimination of the distributions of these cortical projection neurons.

3.) Behavioral testing of rats which received homotopic or heterotopic fetal cortical transplants during development indicates that rats with heterotopic transplants show a marked somatosensory asymmetry as well as contralateral impairments on forelimb placing tasks, while rats with homotopic transplants fail to show any impairments. This suggests that while homotopic fetal cortical transplants may help ameliorate behavioral deficits following neonatal lesions of the rostral cortex, heterotopic transplants do not, and may in fact exacerbate the deficits, despite their seemingly appropriate projections.

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

Our observations on the trajectory of corticospinal axons in jimpy mutant mice indicate that the neurite inhibitory properties of CNS myelin do not play a major role in axonal guidance. The neurite inhibitory properties of myelin may however be very important for the stabilization of connections once they are formed. Our observations on the distributions of cortical projection neurons in jimpy mutant mice indicate that maturation of function accompanying myelination is not critical to the normally occurring collateral elimination during the development of these projection systems. Our behavioral observations on animals with heterotopic or homotopic cortical transplants suggest that different regions of fetal cortex may not be entirely homologous, in spite of the fact that they can, when transplanted, give rise to similar axonal projections.

Proposed Course of the Project:

During the following year our work will proceed along the following lines:

To determine if the absence of myelin in jimpy affects at all the normally occurring axonal loss in the pyramidal tract.

To complete our study of the behavioral effects of homotopic and heterotopic fetal cortical transplants and to begin to explore in more detail any differences in the axonal projections extended and maintained by homotopic and heterotopic fetal cortical transplants.

To study the development of the major projection of the hippocampal formation, the fornix, and to study cell class specification within its neurons of origin.

Publications:

Journal Articles

O'Leary DDM, Schlaggar BL, Stanfield BB. The specification of sensory cortex: Lessons from cortical transplantation, Exp Neurol 1992;115:121-126.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBERZ

ZO1 MH 01106-01 LNP

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Multimodal Plasticity in Behavioral and Neurobiological Compensation of Early Blindness

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

PI:	Josef. P. Rauschecker	Visiting Scientist	LNP, NIMH
Others:			
	Biao Tian	Guest Researcher	LNP, NIMH
	Robert Gelhard	Bio. Lab Tech	LNP, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Neurophysiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH Animal Center, Poolesville, Maryland 20837

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	.25

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

(a1) Minors

(a2) Interviews

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

This project investigates multimodal compensatory plasticity in animals with early blindness , i.e. the capacity of the brain to reorganize itself when visual function is impaired after birth; functions that are normally carried out by one sense may be partly taken over by other senses. We are studying lid-sutured cats and blinded mice to test their somatosensory or auditory functions with behavioral, anatomical, and physiological methods.

As a sign of somatosensory compensation, we have found that visually deprived cats have longer facial vibrissae than sighted cats. These vibrissae, are used for spatial discrimination, and help blind cats compensate for loss of vision in certain tasks. The same whisker growth observed in lid-sutured cats is found in mice if both eyes are removed after birth. The anatomical whisker representation in the somatosensory cortex of these mice, the "barrel field", is expanded by about one third, as shown with cytochrome oxidase and Nissl staining as well as with glutamic acid decarboxylase (GAD) immunohistochemistry.

Auditory compensation of early blindness can be demonstrated for lid-sutured cats in two ways. Behaviorally, these cats can perform sound localization more accurately than normal cats. Neurophysiologically, many more neurons are found in the superior colliculus of the midbrain tectum which respond to auditory stimuli. The same is true for a region of parietal cortex, the anterior ectosylvian area (AES), which provides a major source of cortical input to the tectum. A part of AES which is normally purely visual is taken over by auditory and somatosensory inputs in visually deprived cats. In addition, the spatial resolution of auditory neurons for sounds presented in different locations ("spatial tuning") is significantly sharper in such animals.

Objectives:

Various observations in our colony of blind cats suggest that there is behavioral compensation of the effects of early blindness. The aim of this project is to verify these initial observations with quantitative methods and find the neural basis of this compensatory plasticity with neurophysiological and anatomical techniques. Studies are undertaken in the anterior ectosylvian cortex (AES) of visually deprived cats, because AES contains visual, auditory and somatosensory representations in close vicinity to each other. We wanted to find out (i) whether a change in the proportions of neurons responsive to different sensory modalities occurs in blind animals and (ii) if the response selectivity of auditory neurons, in particular their spatial resolution or sharpness of spatial tuning, changes as a result of visual deprivation.

Methods:

Behavioral, neurophysiological and neuroanatomical methods were applied to study the above questions. Cats were trained to find their way through an obstacle course in the dark by means of operant conditioning. Performance was assessed in an environment lit with infrared light using video techniques in combination with a computerized method of measuring running times.

Neuroanatomical methods included cytochrome oxidase histochemistry of barrel cortex, as well as GAD immunohistochemistry and standard Nissl staining of cell somata.

Neurophysiological techniques consisted of standard single unit recording and application of auditory, visual and somatosensory stimuli. Some of the recordings were done in acute terminal procedures, other animals were prepared for semichronic recording under anesthesia by implanting a headpost and a recording chamber using aseptic techniques.

Major Findings:

A. Somatosensory Compensation of Early Blindness in Cats and Mice

a) Tactile Compensation of the Effects of Visual Deprivation in the Cat

In working with visually deprived cats over the years we have noted an excessive growth of the facial vibrissae in some of these animals, and we have meanwhile published a quantitative assessment of this observation which is statistically highly signficiant. This project now tried to address the question whether these longer whiskers actually have any behavioral advantage and whether somatosensory compensation of early blindness can indeed be demonstrated at the behavioral level.

Cats lid sutured from birth for several years were trained to find their way through a longitudinal maze. Normal adult cats acted as controls and were tested in the light and in darkness with the aid of an infrared camera system. The maze consisted of 10 small gates in 5 different, variable positions. Electrical contacts signalled when the animals passed through each gate, so running times could be measured very precisely. After an initial training period, cats were given at least 8 test blocks (3 trials each) to determine their performance in the maze task. In the light, the normal control group naturally performed somewhat faster than the blind animals, which had to "feel" their way through the maze, but the difference was not very large. In total darkness the normal cats lost their advantage completely, and the blind animals outperformed the normal ones.

It is clear, therefore, that cats without visual experience can acquire knowledge about spatial relationships and can be trained to solve a spatial learning task. While vision obviously helps to solve such a task, the blind animals, when put in a comparable situation with sighted ones, perform at least as well if not better in such a spatial task. Since auditory cues cannot play

a major role for this and odor cues were ruled out, we conclude that the somatosensory system, and in particular the vibrissae system, helps these blind animals to build a space representation which can be used for spatial orientation.

b) Compensatory Changes in the Mouse Vibrissa/Barrel System After Early Binocular Enucleation

The finding that visually deprived cats have on average longer whiskers than normal cats raises also the question whether there is a corresponding expansion of the whisker representation in the central somatosensory system. Rodents, unlike cats, have an anatomically distinct representation of their vibrissae (the "barrel field") in the somatosensory cortex, which can be visualized with various techniques. We therefore decided to switch species and study the barrel field in mice that we enucleated shortly after birth. Using cytochrome oxidase staining we were able to demonstrate that the barrels are indeed enlarged in enucleated mice as compared to normal littermates.

This finding was corroborated with Nissl staining and with glutamic acid decarboxylase (GAD) immunohistochemistry. We found that those barrels which correspond to the longest whiskers are the ones which expand most after visual deprivation. This fits in well with the finding that the longest whiskers are also the ones which profit most in visually deprived cats.

In order to establish the missing link between the two species, we also measured whisker lengths and diameters in our binocularly enucleated mice. It turned out that both lengths and diameters are enlarged as compared to normal littermates, and this enlargement is very highly significant ($p<0.0001$). What is more, the most significant relative growth occurred again in the positions with the longest vibrissae.

B. Auditory Compensation of Early Blindness in Cats

Another form of compensatory plasticity can be demonstrated for the auditory system. Cats with binocular lid suture from birth can localize sound sources in space with greater accuracy and precision, as we have found earlier. The superior colliculus (SC) in the midbrain tectum is one candidate structure where the neural basis for this behavioral improvement could be sought. Indeed, a four-fold increase of auditory-responsive units is found in the SC of visually deprived cats (Rauschecker and Harris, 1983). The major auditory cortical input to SC comes from the anterior ectosylvian cortex (AES), and this projection is strengthened in visually deprived cats. That part of cortex is itself a candidate structure for compensatory plasticity, because here visual, auditory, and somatosensory maps are located in close vicinity to each other. In addition, AES has also been implicated in the control of orientation behavior and seems to be part of a spatial processing stream in cat auditory cortex.

We have analysed neuronal responses in AES to visual, auditory, and somatosensory stimuli in normal and visually deprived cats. The first major result of this analysis is that the proportions of neurons with different sensory modalities in AES are drastically changed in blind animals: Neurons in the fundus of AES, which normally react exclusively to visual stimuli (area AEV), now responded vigorously to auditory and somatosensory stimuli. The few neurons that could still be driven by visual stimulation were bimodal, responding also to other sensory modalities. No increase of unresponsive units was found.

The second main effect is a change in the spatial tuning characteristics of auditory cortical neurons. In blind cats, auditory spatial tuning for azimuth in neurons of the anterior ectosylvian region was sharpened significantly as compared to normal. A spatial tuning index was calculated as the ratio between minimum and maximum response to auditory stimulation in different azimuthal positions. The distribution of spatial indices for normal and blind cats was significantly different ($p<0.001$, median and Smirnov tests). In addition, the proportion of

omni-directional units was 44% in normal cats and only 14% in visually deprived cats. The half-widths of spatial tuning curves were also significantly reduced in all BD animals. It seems possible that these changes in the cortical AES region form the neural basis for the improvements in sound localization behavior of visually deprived cats and that the changes in the SC are only secondary to these changes in the cortex.

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

The study of vision has become one of the most important approaches to understanding brain function in general. The study of impaired vision, in particular, has helped to understand many brain diseases which had not been well understood and which affect a high percentage of the population in the United States and elsewhere. Cats have become the best model for this research on visual deprivation because they have excellent binocular vision (compared with rodents) and because their reproductive cycle is comparatively short (compared with primates). Cortical plasticity of kittens has in fact become one of the most important tools for studying synaptic mechanisms of learning and memory, which is important, among others, for Alzheimer's disease and other illnesses involving memory impairment.

Our studies on the effects of visual deprivation can be expected to have a wide impact on our understanding of the brain mechanisms that cause changes of cortical function in humans and on its possible treatment. Once the brain changes resulting from visual deprivation are understood, we can work on methods to reverse these changes and understand their generality in neuropsychiatric function. The study of inter-modal plasticity takes this approach one step further, closer to the cognitive level. The mechanisms that underlie the development of a "sonar system in the blind", may apply to people with other sensory or cognitive impairments in an analogous fashion. Development of sensory prostheses based on this new knowledge could be one application. In even more general terms, our experiments are of relevance for the understanding of mechanisms for brain repair and restoration of function after any kind of injury in the central nervous system.

Proposed Course of the Project:

1. Animal studies

In the remaining blind cats we want to focus on their improved abilities to localize auditory targets. One striking and highly robust observation in long-term deprived cats is a completely changed pattern of ear-pinna movements. While normal cats move their ears almost exclusively in the horizontal plane (often one at a time), blind cats make very characteristic exploratory pinna movements in the vertical plane, as well. Both ears always move together, and this is often accompanied by head movements reminiscent of radar scanning. Therefore, our hypothesis is that blind cats develop a passive biosonar system in the audible range, which they use to localize and characterize objects in their environment. The aim of the planned experiments in the remaining cats is to study this biosonar system and explore the brain structures which are responsible for the scanning movements of head and ears and the analysis of biosonar information in the auditory cortex.

Our plan is to record pinna movements in normal and blind cats by attaching search coils to the pinnae and to record from neurons in the superior colliculus and the brain stem while the animals receive different kinds of auditory stimulation. Motor-related and sensory-driven activity will be discriminated. Influences of the pinna movements on spatial receptive fields will be noted. Recording from different parts of auditory cortex in awake blind animals will also be performed, in order to measure the effects of pinna movement on auditory responses there.

2. Human studies

In a related project on blind humans we plan to study compensatory plasticity of the auditory system in collaboration with Dr. Stanley Rapoport's group at the National Institute on Aging using measurements of regional cerebral blood flow (rCBF) in blind human subjects.

Publications:

Journal Articles

Rauschecker JP. Mechanisms of visual plasticity: Hebb synapses, NMDA receptors and beyond, Physiol Reviews 1991;71:587-615.

Rauschecker JP, Egert U, Kossel A. Effects of NMDA receptor antagonists on kitten visual cortex, Int J Dev Neurosci 1990;8:425-435.

Rauschecker JP, Tian B, Korte M, Egert U. Crossmodal changes in the somatosensory vibrissa/barrel system of visually deprived animals, Proc Natl Acad Sci USA 1992;89: 5063-5067.

Book Chapter

Rauschecker JP. How does developmental plasticity relate to the biology of memory? In: Squire LR, Lindenlaub E, eds. Symposia Medica Hoechst "The Biology of Memory" New York: Schattauer: Stuttgart, 1990;111-132.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02252-08 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Behavioral Pharmacology and Toxicology

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

PI:	*W. J. Freed*	*Section Chief*	*NPB, NIMH*
Others:			
	A. Herranz	*Visiting Associate*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
Department of Neurosurgery, Naval Hospital, Bethesda, MD (D. Braun)
Department of Medicinal Chemistry, George Washington University (J. Rzesotarski)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Preclinical Neurosciences Section

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.85	2.0	1.85

CHECK APPROPRIATE BOX(ES)

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

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

This project has been terminated.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02253-08 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

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

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

PI:	*W. J. Freed*	*Section Chief*	*NPB, NIMH*
	M. Poltorak	*Visiting Associate*	*NPB, NIMH*
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	A. Herranz	*Visiting Associate*	*NPB, NIMH*
	H. E. Cannon-Spoor	*Psychologist*	*NPB, NIMH*
	O. Dillon-Carter	*Biologist*	*NPB, NIMH*
	S. Jones	*Biologist*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
Department of Neurosurgery, Bethesda Naval Hospital (R. Heim); RWJMS-UMDNJ, Rutgers Medical School, Piscataway, New Jersey (H. Geller, V. Quinones-Jenab); University of Michigan (J. B. Becker; E. J. Curran)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Preclinical Neurosciences Section

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	1.0	3.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews

_(b) Human tissues

x(c) Neither

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

This study is primarily aimed at transplanting tissues containing catecholamines or L-DOPA (i.e., adrenal medulla, tumor cell, genetically altered cell, and embryonic brain tissue) into the brain. Its purpose is to elucidate the properties of these tissues after transplantation and to elucidate the response of the host brain to the transplanted tissues. Specifically, these experiments employ nonprimate animal models to; (1) develop the techniques of brain tissue transplantation for clinical use in Parkinson's disease; (2) develop brain tissue transplantation techniques which eventually may be applicable to other disorders such as schizophrenia and epilepsy, if and when these disorders become sufficiently understood to permit such applications; (3) develop alternate cell types, such as immortalized neurons, for transplantation into the brain; and (4) elucidate the factors controlling brain development and brain response to injury or impairment, with particular emphasis on the nigrostriatal dopamine system. During the past reporting year, significant progress has been made in these areas.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: The overall objective of this study is to develop brain tissue transplantation as a technique for the repair of localized damage to the central nervous system. More specific objectives are to; (1) develop techniques of brain tissue transplantation which can be applied clinically to Parkinson's disease and to other disorders such as schizophrenia or Alzheimer's disease, if and when these disorders become sufficiently understood to permit such applications; and (2) employ brain tissue transplantation to elucidate the factors that control the development and plasticity of the brain, particularly within the nigrostriatal dopamine system.

Methods Employed: The studies involve surgical, behavioral, and histological- histochemical procedures in animal subjects.

Major Findings: Grafts of embryonic substantia nigra (SN) or young adult adrenal medulla have been shown to decrease rotational behavior consequent to unilateral lesions of the substantia nigra. The SN grafts produce dopamine, reinnervate the host caudate-putamen, and decrease spiroperidol binding in the striatum concomitant with their behavioral effects. The adrenal medulla grafts also produce dopamine but do not reinnervate the host brain, and possibly exert their behavioral effects simply through catecholamine secretion. Although these intracerebral grafts, in many cases, survive indefinitely across major histocompatibility typings, inducing rejection through peripheral sensitization of the host animals has been found to be possible. It should be emphasized that the behavioral effects of both adrenal medulla and SN grafts are relatively limited in magnitude. In general, the SN grafts appear to be restricted in their efficacy because of a limited reinnervation of the host brain. In most circumstances, the effects of adrenal medulla grafts are limited because of limited survival of the grafted cells.

1. Trophic effects of cortical and striatal lesions on substantia nigra grafts: These studies are intended to examine the relationship between properties of the brain mileau as a substrate for neurite extension and development of dopamine- containing fibers derived from SN grafts. In an initial experiment, cortical lesions were found to increase the growth of fibers from grafts into the host brain, but only in the most dorsal part of the striatum, close to the lesioned brain area. Reinnervation of other parts of the striatum was not changed by lesions. Results of a long-term study also support this conclusion. These differences were not due to an anatomical distortion of the brain from the lesions or to other anatomical artifacts. The cortical lesions themselves were also found to reduce rotational behavior by substantia nigra grafts. In another experiment, we found that kainic acid lesions of the striatum also stimulated reinnervation of the striatum by substantia nigra grafts. In contrast to cortical lesions, kainic acid lesions of the striatum did not interfere with the behavioral effect of grafts. Studies of cell survival in substantia nigra grafts and effects of other types of lesions are continuing. In these studies, we are now exploring the relationships between changes in the expression of extracellular and cell surface proteins and the extension of dopaminergic neurites from substantia nigra grafts.

2. Combined substantia nigra and striatal grafts: A study of the effects of transplanting combined grafts of substantia nigra and embryonic striatum into the lateral ventricle has been performed. The substantia nigra grafts were found to completely innervate the embryonic striatal grafts in preference to the host brain. When a striatal graft was present in the lateral ventricle, little or no innervation of the host striatum occurred. This study suggests that the mature denervated striatum is a relatively inferior target when compared to the immature striatum. It also suggests that the limited efficacy of substantia nigra grafts is due to properties of the target tissue rather than to a limited efficacy of the substantia nigra

grafts themselves. This paradigm provides us with an additional method for examining the role of extracellular and cell surface proteins in the extension of neurites from transplanted cells. Because both targets are present in the same brain section, the relationship between neurite extension and protein expression can be readily measured.

3. Grafts in neonatal hosts: To exploit the favorable properties of embryonic striatum as the target tissue for substantia nigra grafts, a paradigm was devised to transplant embryonic substantia nigra into the lateral ventricles of normal newborn rats within one day after birth. (Control animals received sciatic nerve grafts.) The animals were then allowed to grow to maturity, and they received bilateral lesions of the substantia nigra. The presence of neonatally implanted substantia nigra grafts protected the animals against development of aphagia, adipsia, akinesia, and rigidity induced by the SN lesions. Differences between substantia nigra-grafted rats and controls were substantial. For example, rats with substantia nigra grafts were 3.7 times as active as the controls. Surviving grafts were consistently found to be well-incorporated into the host striatum. Therefore, the effectiveness of substantia nigra grafts can be increased by transplantation into neonatal hosts. Subsequent studies found that neonatal substantia nigra grafts did not increase behavioral activity of animals without substantia nigra lesions; in addition, these grafts did not alter numbers of surviving, host tyrosine hydroxylase immunoreactive neurons. Using the same paradigm we used for substantia nigra grafts, we investigated the effects of adrenal medulla grafts implanted into host animals on the first day after birth. These grafts had a slight, protective effect against subsequent bilateral substantia nigra lesions, but this effect was much less substantial than that produced by embryonic substantia nigra grafts. These data suggest that the enhanced effects of substantia nigra grafts in neonatal animals are due to a specific interaction between embryonic substantia nigra and the immature host brains. We expect that similar circumstances may be present in immature and damaged brain, and that both can similarly promote dopaminergic neurite extension. Future experiments on this topic will be designed to examine the latter possibility.

4. Trophic effects on intraparenchymal adrenal medulla grafts: Efforts have been directed at assessing the trophic effects and implantation techniques for intraparenchymal grafts of adrenal medulla. Current studies have included evaluating the effects of co-implantating adrenal medullae with other tissues, including peripheral nerve tissue, largely because of the existence of clinical studies on this technique at several universities. These studies are ongoing. Other experiments have examined the effects of other factors including extracorporal time, effects of host age, and changes in graft survival with time after transplantation.

5. Catecholamine release from adrenal medulla grafts: Studies to measure catecholamine release from brain grafts using intracerebral dialysis probes have been initiated in collaboration with Dr. Jill Becker of the University of Michigan. Data obtained so far suggest that catecholamines released from adrenal medulla grafts in the lateral ventricle are not "washed away" in the cerebrospinal fluid.

Adrenal corticosteroids are known to influence the differentiation of adrenal chromaffin cells. Studies on the development of adrenal medulla grafts under conditions of varying, steroid hormone concentrations have therefore been initiated. Results so far suggest that an adrenalectomy does not alter the efficacy of adrenal medulla grafts. No histological correlate of this difference has yet been detected. These data clearly suggest that the presence of the host adrenal gland is not necessary for behavioral efficacy of adrenal medulla grafts. However, the adrenal gland did appear to have a non-specific effect in decreasing rotational behavior in all animals following surgery, including controls that did not receive grafts.

One interesting finding was that blood concentrations of dopamine were related to the degree of behavioral efficacy of these grafts. This accounted, however, for only a part of the effect and was seen only in animals with intact adrenal glands.

Additional studies have shown that blood norepinephrine and epinephrine are not greatly altered by brain adrenal medulla grafts, but blood dopamine is increased roughly four-fold. The increases in blood dopamine were correlated with the behavioral effects of adrenal medulla grafts. This correlation was not present in adrenalectomized animals. Blood catecholamines from peripheral sources seem to be responsible for most of these changes. Thus, it seems that the behavioral effects of adrenal medulla grafts are due to a combination of the non-specific changes in blood dopamine concentrations consequent to surgery, and to a specific effect of adrenal medulla grafts. The specific effect may be mediated by trophic phenomena, by dopamine secretion, or by some combination of the two.

6. Effects of embryonic age on substantia nigra grafts: Recent published studies of human embryonic substantia nigra grafts in rat hosts suggest that very early gestational tissue may be required for behavioral efficacy. However, these studies are inconclusive because of immunological and, possibly, trophic differences between the rat hosts and the human donors. There has, until recently, been no direct study of optimal donor ages in a rat allograft model. We have investigated substantia nigra ion of host animals to established brain allografts: It has become fashionable to suggest that the so-called immunological privilege of the brain does not exist, or that brain grafts survive only because brain tissue is not immunogenic. Previous studies have shown that rejection of established brain allografts can be precipitated by peripheral skin grafts. This may, however, occur only because the generalized immune reaction to grafts induces MHC expression in the brain allografts, subsequently causing rejection of the brain grafts. We therefore wished to determine whether the usual absence of rejection of brain grafts is due to unusual properties of the grafted tissues or to their environment within the brain. Attempts were made to provoke rejection of established brain grafts by peripheral (subcutaneous) brain grafts and by peripheral administration of skin and brain tissue with and without adjuvant. Data collected so far suggest that the major factors influencing brain graft survival are the properties of the host immune system, which are as yet undefined. The specific donor-host combination and other factors, including MHC expression, seem to be less important. Most surprisingly, at least one rat strain (Brown Norway) did not reject MHC-incompatible grafts even after extensive systemic sensitization with donor antigens.

8. Intracerebral transplantation of immortalized glial cell lines: In collaboration with Dr. Herbert Geller of Rutgers University, New Jersey, we are investigating the properties of the A7 line of astrocyte-like glial cells, immortalized by inserting the gene for SV40 large T antigen into primary rat astrocytes in tissue culture and following intracerebral transplantation. Implanted cells were identified by bisbenzimide labelling in combination with immunolabelling. The grafts did not produce neoplasma, and only a fraction of the grafted cells survived. There were no significant immunological reactions to the grafts. The grafted cells retained their in vitro antigenic properties including the expression of N-CAM. Applications of these cell grafts to models of functional recovery sensitive to astrocyte grafts are being developed.

9. Tyrosine hydroxylase-producing cell lines: In collaboration with Dr. Steve Paul, Dr. Ed Ginns, Dr. Sandy Cottingham and others, we are investigating the properties of modified cells containing the enzyme tyrosine hydroxylase, after transplantation. Tyrosine hydroxylase (TH) catalyzes the rate-limiting step in catecholamine and L-DOPA synthesis. The cDNA for form 2 of human tyrosine hydroxylase was used to infect NIH-3T3 mouse fibroblasts, resulting in a stable line of cells containing TH mRNA (TH-3T3 cells). These cells were transplanted into the corpus striatum of Swiss-Webster mice. Intraperitoneal injections of L-tyrosine increased behavioral activity after reserpine treatment in animals that received TH-3T3 cells

as compared to controls in some experiments. In other experiments, no differences were found. The transplanted cells survived and expressed tyrosine hydroxylase mRNA after transplantation. Additional methods and development of additional cell types are being pursued.

10. Development of immortalized neurons: We are in the process of immortalizing embryonic, mesencephalic, and striatal neurons using the temperature-sensitive variant of SV40 large T antigen. The resultant cells will be used for in vitro studies of neuronal differentiation as well as for experimental transplantation. We have presently initiated approximately 40 cultures which have been exposed to the disabled virus. We have obtained approximately 5 replicating colonies of neuron-like cells. These colonies have not yet been cloned or exposed to G418.

11. Role of substrates in differentiation of dopaminergic neurons: We have also been employing in vitro methods to examine the effects of various substrates of in vitro extension of neurites from embryonic, dopaminergic neurons. We have found that L1/NgCAM is the most potent substrate, followed by fibronectin and laminin. Other molecules, including L2, meylin-associated glycoprotein, poly-L-lysine, and RGD attachment peptide, were less effective or ineffective. These studies will be compared with in vivo experiments to define the role of extracellular matric and cell surface protein expression in the differentiation and growth of dopaminergic neurites.

Proposed Course of Project: Investigation of brain tissue transplantation as a therapeutic technique applicable to Parkinson's disease is expected to continue. Subsequently and concurrently, grafting will be studied as a means of assessing trophic control of the development and function of brain dopaminergic systems. Studies of possible applications of brain tissue transplantation to other disorders may also be initiated as developments in other fields elucidate possible applications.

Significance to Mental Health Research: These studies may lead to the development of brain tissue transplantation as a therapeutic procedure for Parkinson's disease and eventually for other disorders. In addition, brain tissue transplantation is a valuable technique for the investigation of trophic functions in the brain. For example, the finding that brain injury has a trophic effect on dopamine-containing neurites is of potential importance for understanding the developmental and trophic influences on the brain's dopaminergic system and its possible dysfunction in schizophrenia. Subsequent studies using this paradigm may provide useful information relating to the effects of brain injury on neuronal circuits. Investigation of trophic functions and their possible absence is of particular importance for diseases such as schizophrenia, which may involve relatively subtle forms of neuronal dysfunction rather than readily detectable brain atrophy or neuronal degeneration.

Publications:

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Simmonds GW, Schwarz S, Krauthamer E, Freed WJ. Effects of adrenal medulla grafts in neonatal rat hosts on subsequent bilateral substantia nigra lesions, Restorative Neurology and Neuroscience 1990;1:315-22.

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Dymecki J, Poltorak M, Freed WJ. The degree of genetic disparity between donor and host correlates with survival of intraventricular substantia nigra grafts, Reg Immunol, in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02257-08 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemical and Neuroradiologic Abnormalities in Tardive Dyskinesia

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

PI:	*M. F. Egan*	*Sr. Staff Fellow*	*NPB, NIMH*
Others:			
	A. Elkashef	*Commissioned Corp*	*NPB, NIMH*
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
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LAB/BRANCH
Neuropsychiatry Branch

SECTION
Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

x(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.)*

We have replicated the results of a preliminary outpatient study showing that treatment with alpha-tocopherol (vitamin E) decreases symptoms of tardive dyskinesia (TD). This supports the free radical hypothesis of TD. We are planning several additional studies to further explore the neurobiology of TD. These include trials of medications, neuropsychological tests, neuroradiological examinations, and evaluations of eye tracking. We are studying the following three hypotheses: (1) free-radical scavengers reduce the severity of and prevent the development of TD; (2) TD is associated with neuronal cell loss in the striatum; and (3) cognitive impairment is associated with TD in older but not younger patients relative to patients without TD.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: Tardive dyskinesia (TD) is a significant public health problem affecting 20 to 40 percent of patients treated chronically with neuroleptics. A recent review of the literature shows no proven effective treatment. Palliative relief can be obtained by using higher doses of neuroleptics, but this carries the risk of exacerbating the disorder in the long run. We have recently replicated a preliminary study which found that, early in the illness, alpha-tocopherol was effective in ameliorating the symptoms of TD. The purpose of these studies is to further explore the neurobiology of TD, and, in particular, to look for evidence of neurodegeneration. This will be done in several ways. First, a clinical trial of a potent antioxidant, coenzyme Q, will be initiated in patients who have had TD for five years or less. Second, MRI scans of patients with TD will be obtained to look for shrinkage in the basal ganglia, relative to patients without TD. Third, patients with TD will undergo a number of neuropsychological tests, and will be compared to patients without TD to look for evidence of specific cognitive deficits. Eye tracking will also be obtained in many of these patients. Finally, using an animal model of TD, we will look for evidence of neuronal cell loss and other neurochemical changes in the striatum.

Methods Employed:

1) Clinical Studies: Coenzyme Q (a potent antioxidant) will be administered to patients with TD in a double-blind, placebo-controlled crossover study. Patients will be recruited from the usual NIMH national sources as well as from a TD Clinic at St. Elizabeths created specifically for this purpose. Patients will be videotaped weekly during an AIMS examination. These exams will be rated at the end of the study by blind raters. Patients will also receive neuropsychological testing during both treatment periods to look for evidence of cognitive improvement. The Psychiatric Symptom Assessment Scale and Negative symptom Rating Scale will also be employed to assess changes with treatment in psychopathology. Eye tracking will be done in collaboration with Dr. Bruce Roberts of the Laboratory of Socio-Environmental Studies. MRI scans will be performed at the Clinical Center. Measurements of subcortical structures will be done using a computerized image analysis program.

2) Animal Studies: Rats with a neuroleptic-induced, persistent movement disorder will be used for postmortem studies. We will initially use immunocytochemistry to look at GAD staining in the striatum. In collaboration with the neuropathology laboratory in the Clinical Brain Disorders Branch, we plan to use *in situ* hybridization to look at changes in postsynaptic spiney II GABAergic neurons in the striatum. We anticipate alterations in the expression of GAD, enkephalin, and Substance P. We hypothesize that there will be a significant decrease in the number of cells expressing these peptides in animals with dyskinesias.

Major Past Findings: In a recently completed, inpatient study, 18 patients treated with vitamin E did not show a significant improvement. However, when the patient group was divided in half, based on duration of TD, patients with TD for 5 years or less showed a modest, but significant, improvement of 18.5% in AIMS ratings. This is similar to two previous studies where patients on vitamin E also improved. In these two studies, the average duration of TD was similar to our group with TD for 5 years or less.

Significance to Mental Health Research: Tardive dyskinesia affects 20 to 40 percent of psychiatric populations treated with neuroleptics. This iatrogenic illness is at times disfiguring, leading to difficulty in reintegrating the chronically mentally ill into society. Furthermore, TD itself can progress to the point of causing impairment in chewing, swallowing, and breathing, leading to significant increases in morbidity and mortality. Since no good treatment exists, any advance in understanding the pathophysiology of the disorder or its treatment will have a great impact on patient care and future research. Three studies have shown that alpha-tocopherol may be effective, implying that free radical formation and the resulting neuronal injury may be important in the genesis of the disorder. Treatment with more potent antioxidants may provide additional benefits.

Proposed Course of Project:

(1) Twenty patients will be enrolled in a double-blind, placebo-controlled crossover study of 12 weeks duration using coenzyme Q. We expect to enroll 6 to 8 patients per year over the next 2 1/2 years.
(2) Both inpatients and outpatients attending the TD clinic will be recruited for neuropsychological testing and MRI scans. We expect to include 10 schizophrenic patients with and 10 without TD each year for the next 2 years.
(3) Animal studies. Animals will be given coenzyme Q concurrently with haldol decanoate for six months to see if coenzyme Q reduces the incidence of TD. Postmortem neuropathological studies will be conducted comparing levels of various transmitters, peptides and enzymes in the striatum between animals with and without TD.

Publications:

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Khot V, Korpi E, Venable D, Wyatt RJ: Neuroleptics and classical tardive dyskinesia, Progress in Movement Disorders, in press.

Egan M, Hyde T, Albers G, Elkashef A, Alexander R, Reeve A, Blum A, Saenz R, Wyatt RJ: Treatment of tardive dyskinesia with Vitamin E, Am J Psychiatry, in press.

Egan M, Hyde T, Tirschwell D, Kleinman J, Weinberger D: Laterality of Appendicular Tardive Dyskinesia in Chronic Schizophrenia, Biol Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02259-08 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Peripheral and Central Catecholamine Turnover in Mental Illnesses

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

PI:	*F. Karoum*	*Research Chemist*	*NPB, NIMH*
Others:			
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Molecular Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews

x(b) Human tissues

_(c) Neither

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

Combined gas chromatographic mass spectrometric methods have been employed to assess total body turnover of norepinephrine (sum NE) and dopamine (sum DA) in human subjects and rats. (1) We have continued to gather additional supportive data that suggest a tendency for sum NE to be elevated in major depression, and have also observed a positive correlation between urinary-free cortisol, and urinary NE and vanillmandelic acid (VMA), a major metabolite of NE in the periphery in humans. (2) Total body NE and DA turnover was assessed in both hyperactive children and adults after several pharmacological manipulations. The results indicated a correlation between therapeutic benefits and changes in both sum NE and DA irrespective of the direction of change. (3) The effects of 4 commonly used antidepressant treatments (chronic zimelidine, desipramine, electroconvulsion, and lithium) on rat peripheral and central catecholamines were evaluated. A good correlation between the effects of these drugs and sum NE and sum DA in humans and rats was observed. It is suggested that because of this correlation, changes in the rat brain amines probably resemble the changes these treatments induce in the human brain. (4) We are currently attempting to reproduce our initial study on DA and NE turnover in schizophrenia, and hope to also include patients with tardive dyskinesia. (5) The above procedures were employed in the evaluation of central and peripheral production of catecholamines in Norrie Disease.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: To assess and determine the role of peripheral and central catecholamines and other biogenic amines in mental illnesses.

Methods Employed: All biochemical analyses were performed by combined gas chromatographic mass spectrometric methods developed in this laboratory.

Major Findings: We have continued to employ the approach described in the 1987 Annual Report: to evaluate the total body turnover of catecholamines in depression and schizophrenia. This approach has been extended to evaluate total body turnover of catecholamines in the experimental animal following various pharmacological manipulations. We hope to correlate our findings in this latter investigation with the dynamics of the drugs transported from the periphery into the brain and with the profile of the drugs' metabolism.

In Norrie Disease patients with a partial X-chromosome deletion, vanillymandelic acid (VMA) and 3-methyoxy-4-hydroxyphenylglycol (MHPG) excretion were extremely low compared to patients without X-chromosome deletion. Furthermore, patients with partial X-chromosome deletion showed large (over 100-fold) increases in phenylethylamine excretion.

These findings suggest the absence of monoamine oxidase (MAO-A and MAO-B) in patients with X-chromosome deletion. It is therefore possible that the portion of the X-chromosome deleted in these patients carries the gene encoding for MAO-A and MAO-B. This conclusion is consistent with reports of the localization of the gene encoding for the expression of MAO-A and MAO-B on the X-chromosome in humans. In contrast to norepinephrine metabolites, dopamine and 5HT metabolites were normal in patients with partial X-chromosome deletion, thus indicating that types of MAO-A different from those on the X chromosome are responsible for the catabolism of dopamine and serotonin.

Significance to Mental Health Research: Our findings, which are related to total body turnover of catecholamines in depression, schizophrenia, hyperactivity in children, and Norrie disease, have convinced us that the methods employed are useful in studying the role of catecholamines and other biogenic amines in mental illnesses.

Proposed Course of Project: We continue to use rats to correlate total body amine turnover with central amine turnover and metabolism. Our methods accurately and reliably measure a variety of biogenic amines in biological materials so we include, whenever necessary, information on the disposition of such amines as

phenylethylamine, tyramine, and the indoleamines. In addition, we are pursuing the correlation of changes in catecholamine turnover in both human subjects and experimental animals with drug concentrations in plasma, urine, and other biological media.

Emphasis in all future investigations will be directed towards understanding the mechanisms of action of drugs on both central and peripheral aminergic systems. In these studies we plan to carry out simultaneous measurements of plasma and CSF concentrations of biogenic amines and their metabolites in schizophrenics while on and off neuroleptics.

Publications:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02263-08 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Haloperidol Pharmacodynamics and Clinical Response in Schizophrenia

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

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Others:			
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	L. Wing	*IRTA Fellow*	*NPB, NIMH*
	R. Li	*Visiting Fellow*	*NPB, NIMH*
	R. Suddath	*Sr. Staff Fellow*	*NPB, NIMH*
	F. Issa	*Commissioned Officer*	*NPB, NIMH*
	A. Elkashef	*Commissioned Officer*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
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LAB/BRANCH
Neuropsychiatry Branch

SECTION
Clinical Neuropsychiatry

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

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x(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.)*

As part of a standardized sequence in the NIMH intramural clinical research program at the Neuroscience Center at Saint Elizabeths, patients with schizophrenia are withdrawn from neuroleptic medication and, after clinical relapse, treated with a fixed dose of haloperidol. This in turn allows a variety of studies regarding the pharmacokinetics of haloperidol and the effects of haloperidol and other neuroleptics on central nervous system catecholamines. In addition, the response of these patients to alternative, non-neuroleptic antipsychotic agents is studied. Other pharmacological phenomena being examined in these patients include drug-drug interactions involving haloperidol and several other drugs, especially nicotine and caffeine. The direct effects of haloperidol, nicotine, and caffeine on central catecholaminergic neurotransmitter/receptor function, cellular second messengers, and behavior are also being studied in both clinical and basic science investigations. A final focus is on elucidating the mechanisms involved in the syndrome of polydipsia-hyponatremia, a potentially lethal problem that is relatively common in neuroleptic-treated patients.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: Major goals of the project include elucidating the relationship between serum haloperidol concentrations and clinical variables, the pharmacokinetics of haloperidol when used in chronic treatment, and drug-drug interactions between haloperidol and two other drugs commonly consumed by schizophrenic patients, nicotine and caffeine. A key step in defining these relationships is the characterization of the effects of haloperidol and other drugs, alone and in combination, on central nervous system (CNS) monoamines, second messenger systems, and behavior. Research to clarify the impact of nicotine and caffeine use on patients with chronic schizophrenia and tardive dyskinesia is also being conducted. Studies of non-neuroleptic agents are conducted in order to identify alternative drugs which might have antipsychotic effects. Lastly, the problem of polydipsia-hyponatremia, a relatively common and potentially lethal clinical syndrome that is seen in patients with schizophrenia and that may be related to drug treatment, is being studied in order to define better its pathogenesis and treatment approaches.

Methods Employed: Research subjects who come into the Neuropsychiatric Research Hospital at the NIMH Neuroscience Center at Saint Elizabeths are placed on coded neuroleptics in a standardized protocol. They are treated with either active fixed-dose haloperidol (0.4 mg/kg/day) or placebo in a double-blind fashion. This allows acute-dose and withdrawal pharmacokinetic and steady state samples to be collected for serum haloperidol concentrations. Moreover, haloperidol can be measured while the patients are being treated with other drugs, including the nicotine and caffeine frequently consumed in large amounts by these patients. Blood samples are also collected from this group for quantification of nicotine, cotinine (a nicotine metabolite), and caffeine. After establishing the response to haloperidol, the clinical effects of other drugs may also be assessed. Other clinical cohorts have been studied during haloperidol treatment: an international multi-center patient group coordinated by the World Health Organization (WHO) and Dr. Potkin; and a group of acute patients (including elderly subjects) coordinated by Dr. Hertzman at George Washington University.

The pathophysiology of polydipsia-hyponatremia in these schizophrenic patients is being studied by assessment of fluid balance on and off neuroleptics, behavioral and pharmacologic treatment approaches (including treatment with demeclocycline, a peripheral vasopressin antagonist), and magnetic resonance imaging.

The method of quantification of haloperidol and reduced haloperidol concentrations in our laboratory involves high performance liquid chromatography (HPLC) after a liquid-liquid extraction process. Monoamines are

also measured in our laboratory using an exceptionally sensitive HPLC system coupled with dual-electrode electrochemical detection. Access has also become available thorugh Dr. Gerhardt to a new multielectrode detection HPLC system that allows simultaneous quantification of multiple amines, amino acids, and small peptides. This method has great promise in performing multivariate biochemical profiles of human cerebrospinal fluid (CSF) samples. Studies of phosphoinositide turnover by Dr. Li have used a phosphoinositide hydrolysis assay in which brain slices are incubated with myo-[2-^{3}H]inositol and incorporation of this compound into phospholipid is quantified. Nicotine, cotinine, and caffeine have been measured by Dr. Benowitz using gas chromatography.

Basic science animal studies are being conducted in rats by Dr. Wing using the chronic administration of haloperidol and other drugs by either injection or infusion via osmotic mini-pumps. This allows a pattern of chronic exposure similar to that encountered in schizophrenic patients. Multidimensional behavioral monitoring is performed in these animals using automated activity monitoring boxes.

Major Findings: Single cell recordings in rats showed that reduced haloperidol, a metabolite of haloperidol, appears to be pharmacologically inactive. Studies of the relationship between serum haloperidol concentration and clinical response indicate that, above a threshold concentration (which appears to be approximately 5 ng/ml), patients have a relatively constant degree of clinical improvement up to serum concentrations of 40 ng/ml. Thus, our own data failed to support the concept of a "therapeutic window" for haloperidol that has been proposed by some other investigators. To replicate the earlier finding regarding the relationship between serum concentrations and clinical response, Dr. Potkin coordinated a multisite WHO study in which patients were given fixed doses of haloperidol, serum concentrations were measured, and clinical responses were assessed. Initial analyses of the data regarding clinical response at 4 weeks again showed that, above a certain threshold, patients had a comparable response and there was no evidence of a "therapeutic window." Further study of NIMH patients indicated that red blood cell haloperidol concentrations are no better predictors of clinical response than plasma concentrations. The pharmacokinetics also indicated a relative preferential uptake of reduced haloperidol into red blood cells, indicating that tissue stores of this reduced metabolite may be high. Data (submitted for publication) regarding haloperidol treatment of acutely ill psychiatric patients across a wide age range at George Washington University Medical Center showed that older patients developed significantly higher concentations of haloperidol in response to a given dose compared with younger patients, highlighting the clinical need, to adjust haloperidol doses downward in this population that is exceptionally vulnerable to side effects.

Pharmacokinetic data were accumulated regarding the response to an acute dose of haloperidol and the washout from haloperidol after its withdrawal. Data analyses showed a peak in serum concentration 3 to 5 hours after acute administration, with a highly significant correlation between the acute dose response at 8 hours and ultimate steady state concentration. Withdrawal data showed an initial half life of less than 24 hours, with a possible secondary phase of much slower drug elimination. Smoking was shown to be associated with lower serum haloperidol concentrations. There was no evidence that tardive dyskinesia was associated with increased haloperidol concentrations.

A study of the combined effect of haloperidol and ascorbic acid indicated that the latter compound does not affect serum haloperidol concentrations and does not appear to enhance clinical response in patients with chronic schizophrenia.

Basic science data showed a significant pharmacokinetic interaction between haloperidol and retinoic acids, both in serum and in central nervous system tissue.

Plasma catecholamine metabolites in samples from patients before and after withdrawal from neuroleptics showed a trend toward an increase in homovanillic acid (HVA) and a significant increase in 3-methoxy-4-hydroxyphenylglycol (MHPG). Further follow-up assessment of patients evaluated earlier with Dr. Freedman has indicated that the presence of high plasma levels of the dopamine metabolite, homovanillic acid, may be associated with poor long-term prognosis.

Our studies involving nicotine showed that patients diagnosed as schizophrenic are more likely to smoke than chronically hospitalized patients with other diagnoses. A larger epidemiologic survey confirmed increased smoking among patients with schizophrenia compared with other diagnoses. Moreover, smokers who have schizophrenia were more likely to have tardive dyskinesia than those who do not smoke. It was also found that patients with tardive dyskinesia have significantly higher plasma concentrations of caffeine and a tendency toward higher nicotine concentrations (as measured by Dr. Neil Benowitz) than patients without dyskinesia.

A rat study, in collaboration with Drs. Gerhardt and Freedman, regarding nicotine showed a decrease in dopamine turnover in the striatum, frontal cortex, and hypothalamus when rats were treated with nicotine for a period of 3 weeks. Basic science studies of the combined effect of haloperidol and nicotine on rat brain catecholamines indicated that the combination of these drugs has a significant synergistic effect on CNS monoamines. Caffeine was also shown to significantly affect monoamine turnover in the rat.

Work done with Dr. Creese regarding the effects of nicotine and caffeine on catecholamines indicated that neither drug causes a significant change in D1 or D2 receptors in rat striatum with chronic administration. This, in turn, implies that the apparent positive association between nicotine, caffeine, and tardive dyskinesia may be due to some mechanism other than receptor upregulation.

A study of the effects of a calcium channel inhibitor, nifedipine, in schizophrenic patients showed that, while there was no apparent antipsychotic effect, there was a significant decrease in the severity of abnormal movements in those patients with tardive dyskinesia.

Turning to studies of polydipsia-hyponatremia, our trial using demeclocycline to treat polydipsia-hyponatremia showed no significant beneficial effect of the drug in the treatment of this syndrome.

New Findings: Using the new multielectrode detector HPLC system, Dr. Gerhardt has now completed an extensive analysis of CSF samples from schizophrenic patients and controls. Measures of 19 different compounds were performed in the CSF samples and, because of the large number of interrelated variables, the data were analyzed by Dr. Issa using stepwise discriminant function analyses. Comparison of patients versus controls showed tryptophan, tryptophol, and epinephrine to be discriminating variables. A matched pairs analysis in patients on versus off medication showed no significant differences. When the outcome of the treatment on the fixed-dose neuroleptic protocol was compared with CSF amine concentrations, 3-hydroxykynurenine and kynurenine determined off medication predicted the treatment response in a multiple regression model. These data on CSF amines will also be analyzed in relation to structural brain measures determined from MRI scans.

Three studies of drug effects on cellular second messenger systems have now been completed by Dr. Li. The first demonstrated that, in the rat, chronic haloperidol treatment attenuates carbachol-induced stimulation of phosphoinositide turnover. These results, which resemble the effect of lithium on phosphoinositide turnover, were striking insofar as both haloperidol and lithium are effective treatments for mania. This led to a second study (submitted for publication) comparing the effects of haloperidol, lithium, and valproate (another anti-manic agent) on phosphoinositides. The results showed that all three drugs, with some differences in time course and brain regional specificity, have the shared ability to decrease phosphoinositide turnover. This observation may in turn be relevant to their common efficacy in the treatment of mania. In a third study (in press), it was shown that nicotine, a drug preferentially used by patients with a number of psychiatric disorders, may also affect phosphoinositide metabolism when chronically administered to the rat, and may also significantly interact with haloperidol when co-administered.

Dr. Wing has accumulated extensive data regarding the effects of chronically administered haloperidol, nicotine, and caffeine on behavior and brain neurochemistry in the rat. One study (now in revision for publication) confirmed the ability of nicotine to initially potentiate haloperidol-induced decreases in behavior. Both nicotine and haloperidol were shown to have regionally specific effects on brain monoamines after 6 weeks of treatment. Another study compared the behavioral and neurochemical effects of nicotine and the serotonin reuptake inhibitor, fluoxetine. While each drug individually affected monoamine concentrations, there did not appear to be the kind of synergistic interaction as had been observed earlier with nicotine potentiation of haloperidol-induced decreases in behavior. Data are also being analyzed using similar measures looking at the effects of and interactions between haloperidol and caffeine.

Results of an expanded magnetic resonance imaging (MRI) study of patients with polydipsia-hyponatremia conducted with Dr. Suddath confirm that these patients have larger lateral ventricles and significant decreases in tissue volumes in medial temporal structures, especially the anterior hippocampus and amygdala (regions thought to be relevant to drinking behavior), compared with other schizophrenic patients who do not have polydipsia and hyponatremia. In a parallel finding, analysis of data regarding endocrine function in response to a trial of treatment with a vasopressin analogue, indicates that patients with larger lateral ventricles on computed tomographic brain scans were more vulnerable to developing the complication of clinically significant hypontremia in response to the drug, supporting the idea that CNS structural damage may be in some way linked to the propensity to become hyponatremic in these patients. An ongoing study of polydipsic-hyponatremic patients conducted by Dr. Elkashef using MRI scans done before and after fluid loading is confirming that these structural brain changes are not state-related. In fact, the process of fluid loading appears to increase tissue volumes (perhaps reflecting edema), a fact which makes the observed decreases in tissue volume in polydipsic-hyponatremia patients more striking.

Significance to Mental Health Research: Haloperidol remains one of the most commonly used drugs in the treatment of schizophrenia, and for research purposes it remains very much a prototype neuroleptic with a relatively uncomplicated metabolism. The data produced by this project help provide a more rational strategy for dose selection, elucidate important drug interactions, and may clarify the role of alternative antipsychotic drugs.

The studies of neuroleptic effects on catecholamines and second messengers are directed at clarifying the basic CNS neurochemistry involved in schizophrenia and the response to neuroleptic treatment, as well as seeking explanations for the overlap in psychotherapeutic effect of drugs from different classes (e.g., haloperidol and lithium).

The studies regarding nicotine and caffeine, as well as their interactions with haloperidol, not only may increase our understanding of why patients with schizophrenia are so prone to use these drugs, but also may reveal information on the mechanisms underlying dependence on these substances in normal individuals.

The syndrome of polydipsia-hyponatremia is relatively frequent and can be lethal. It is important to understand its pathophysiology to either prevent it or develop effective interventions.

Proposed Course of Project: The haloperidol pharmacokinetic studies are now focused mainly on final revisions in response to reviewers' comments of the manuscripts regarding acute dose and fall-off pharmacokinetics, effects of age on serum concentrations, and associations between neuroleptic concentrations and both smoking and tardive dyskinesia.

With regard to studies of monoamines, amino acids, and peptides in patients, the data obtained from the analysis of CSF using the HPLC system with multichannel detection have been analyzed and manuscripts are being submitted. The next analysis will focus on the associations between amine measures and MRI structural volumes, testing the hypothesis that cerebral structural abnormalities may be associated with monoamine abnormalities, especially lower CSF homovanillic acid. Dr. Gerhardt has also agreed to pursue in more detail the assay of multiple compounds in the kynurenine pathway, such as quinolinic acid, which have been implicated in degenerative central nervous system disorders.

Dr. Li will pursue our finding regarding a common effect of psychotropic drugs from diverse classes on phosphoinositide turnover. We will seek to determine the relative role of receptor systems, especially D_1 and D_2 dopamine receptors, in these phosphoinositide alterations.

The basic science studies by Dr. Wing of nicotine and caffeine are focusing on the effects of these drugs on monoamines and animal models of behavior, with multiple manuscripts in preparation.

The study of polydipsia-hyponatremia will focus on expansion of the number of subjects studied by magnetic resonance imaging, including the study by Dr. Elkashef of within-day changes in brain structure related to fluid-loading in polydipsic patients.

Publications:

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Kirch D, Wing L, Li R, Wyatt R. Nicotine and tardive dyskinesia. In: Racagni G, Brunello N, Fukuda T, eds. Excerpta Medica, Amsterdam, 1991; 621-23.

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Li R, Wing L, Kirch D, Wyatt R, Chuang D. Effects of chronic nicotine and haloperidol administration on muscarinic receptor-mediated phosphoinositide turnover in rat brain slices, Psychopharmacology, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02275-08 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Search for Virus in CSF and Post-Mortem Brain of Patients with Schizophrenia

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

PI:	*J. R. Stevens*	*Medical Officer*	*NPB, NIMH*
Others:			
	H. Kulaga	*Research Biologist*	*NPB, NIMH*
	S. Shirabe	*Stanley Fellow*	*NPB, NIMH*

COOPERATING UNITS (if any)
NINDS, NIH (D. Asher, J. Schwartz)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Aging

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	1.00	.25

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  _(a2) Interviews

x(b) Human tissues

_(c) Neither

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

Epidemiologic, immunologic, and pathologic evidence has suggested that, in a significant number of cases, schizophrenia may be related to infection with or activation of a viral disease of the brain. To pursue this possibility, we previously undertook immunocytochemical and in situ hybridization studies of brain in which we sought evidence of specific viral antigens or genomes. With rare exceptions, these studies did not disclose viral antigens. Intracerebral inoculation of schizophrenic brains of guinea pigs and non-human primates also yielded negative results. Two new approaches have since been undertaken: (1) Co-cultivation of schizophrenic or control CSF lymphocytes with human neuroblastoma cells (SH-EP), and (2) Intracerebral inoculation of control or schizophrenic lymphocytes in newborn mice. The best study demonstrated growth to a higher density of SH-EP cells treated with CSF cells (freshly drawn) from 10 of 12 chronic schizophrenic patients from the NIMH unit at St. Elizabeths Hospital.

In an attempt at replication, a spundown aliquot of CSF from twelve patients with acute or exacerbated schizophrenia, and from 13 age-matched neurologic controls from the Oregon Health Sciences University in Portland, Oregon and cooperating hospitals, were cultured with SH-EP human neuroblastoma cells. In contrast to the SEH material, all of the Oregon cultures, treated with schizophrenic and with control CSF, grew to the same high density. We attributed this to changes in culture medium and environment, and launched a 3d study with patients from SEH and schizophrenic twins. Five schizophrenic patients and 0 controls have transformed SH-EP cells. Current work is focussed on cloning and sequencing the nucleic acid responsible for the transformation. We are also repeating the original study of CSF on a new group of Saint Elizabeths' patients and controls.

In the spring of 1988, we inoculated frozen CSF from acute and chronic schizophrenic patients (n=14) and controls (n=13) intracranially in newly born mice (n=107). Systematic behavior tests were done on these animals at two months of age. Animals were sacrificed if sick or at one year after inoculation if no illness developed. No significant changes were found in brains of schizophrenic compared to control mice, with the exception of larger ventricles in mice injected with fresh, but not frozen CSF.

Project Description:

Objectives: The purpose of this investigation is to search of evidence for an infectious or toxic agent as a significant etiologic factor in schizophrenia or a subgroup of schizophrenic patients. This work was stimulated by evidence from a number of sources, including epidemiologic, immunologic, geographic, and neuropathologic studies compatible with an infectious etiology in this disorder. Highlights of the evidence include reported increased immunoglobulin in the cerebrospinal fluid of schizophrenic patients, CSF antibodies to specific viral agents (CMV, HSV); seasonal birth peaks of schizophrenic patients; uneven geographic distribution; abnormal response of lymphocytes to specific mitogens; toxic effects of urine, serum, or cerebrospinal fluid of schizophrenic patients on animal behavior and tissue cultures; and gross and histologic neuropathologic changes in the brains of individuals with schizophrenia. Our previous attempts to identify antigens or viral genome from schizophrenic brains or passage of this disorder to animals or through cell cultures have with a few exceptions been negative. In contrast, our cell culture investigations demonstrated that one line of human neuroblastoma cells grew to a higher density many weeks and passages after incubation with cells for 5 days with 0.1 cc fresh centrifuged CSF subnate of patients with chronic schizophrenia, but not with fresh CSF subnate from controls.

Methods Employed: Two groups of schizophrenic patients and controls have been studied and a third is in progress:

1. Thirteen drug-free patients with chronic schizophrenia from the research wards of NIMH Neurosciences Center at St. Elizabeths and 15 controls (7 patients with neurologic disorders from George Washington University's neurology service and 8 normal controls from SEH staff).
2. Twelve patients with a diagnosis of acute schizophrenia or schizophreniform disorder from Oregon Health Science University's psychiatric admission ward and 13 controls from Neurologic and Neurosurgery Services of the Oregon Health Sciences University and Good Samaritan Hospital, Portland, Oregon.
3. Eight patients with schizophrenia and 8 normal controls from research wards and hospital staff at SEH.

Eight-10 cc of freshly drawn CSF is centrifuged at 300 rpm for 5 minutes, and the spun-down 0.5 ml subnates containing WBCs and 0.5 cc of supernate were separately incubated for 5 days with human neuroblastoma cells (SH-EP) after which cells were fed 3x weekly and passaged at 2 week intervals for 6-12 months.

Major Findings: In the first SEH sample, no cytotoxic effect was observed, but after 1-6 months, neuroblastoma (SH-EP) cultures that had been exposed to fresh CSF from 10 of 13 schizophrenic patients grew to 25-100% higher density than 12 of 15 control-CSF-treated cultures ($p<.01$). Transformation was further evaluated by testing colony

formation in soft agar. Three of 4 schizophrenic-CSF, but only 1 of 13 control-CSF-treated cultures, showed increased size and/or number of colonies. The agent causing growth to increased density and colony formation has continued to be expressed for up to 30 passages, can be transmitted by cell-free media passed through a .45 μm filter, and, in 1 ml aliquots, diluted 1:100. Enhanced growth was expressed more often in cultures exposed to cells (subnate) from schizophrenia-CSF compared with those exposed only to schizophrenia-CSF supernatant. Neuroblastoma cells exposed to schizophrenia-CSF also make more adenylate cyclase than SH-EP cells treated with control CSF.

Evaluations for reverse transcriptase, oligoclonal immunoglobulin, lectin surface markers, abnormal protein in 2D gels, or electron microscopic evidence for viral particles have all been negative in these specimens. Dr. Shirabe has isolated the nucleic acid associated with cell transformation from positive CSF, and is preparing to clone and sequence this DNA, after which it will be used as a molecular probe in stored samples or transformed control cells, brain and CSF.

SH-EP cells incubated with CSF from Oregon controls, acute schizophrenic patients, and untreated cells all grew to a density equivalent to the schizophrenia-CSF-treated cells in the Neuropsychiatry Branch sample, an effect we consider may have been due to culture conditions (different sera) in Oregon. To ascertain that the change in growth was not simply a product of a high number of passages, we repeated this study using the original cell line obtained from Dr. June Beidler. Incubation of this new batch of SH-EP cells with cell free media from the schizophrenia-CSF-treated neuroblastoma cells also caused these low passage neuroblastoma cells to grow to higher density than control CSF-treated cells. This did not happen with control sera.

Part II. Ten μl of sterile frozen CSF from the acute schizophrenic cases from Oregon (n=13), from twins in E.F. Torrey's study of monozygotic twins discordant for schizophrenia (n=6), and from cells from freshly drawn CSF of current St. Elizabeths Hospital Research Unit patients (n=3) and from controls (n=15) were injected intracranially in right frontal lobe of 1-3 day old suckling mice. Half of each litter was inoculated with schizophrenia-CSF and half with control. Animals have been maintained in laminar flow cages, checked for general health at frequent intervals, and given standardized quantified behavior tests at 2-3 months of age. Brains are removed and fixed in formalin from animals that sicken or die. At one year all other animals are sacrificed, brains removed, frozen or fixed, and prepared for histologic examination for inflammation or gliosis. Hydrocephalus has appeared in 3 brains. No pathologic changes distinguished the brains injected with cell-free frozen CSF from patients or controls. Hydrocephalus is over represented in mice inoculated with fresh cells containing schizophrenic CSF.

Significance to Mental Health Research: Schizophrenia is one of the most disabling neuropsychiatric problems in the United States, and, indeed, in the world.

Schizophrenia affects approximately 1 percent of the U.S. population. It is young people who are most affected, and 20-25 percent of those affected are severely and permanently handicapped despite use of the most modern therapeutic measures. New approaches to both prevention and treatment are urgently required. Because of circumstantial evidence for infection as a significant cause of some schizophrenias, this effort is focused on the search for an etiologic agent. We are fully aware that this is "long-shot" research with no immediate promise of answers. The cell transformation observed in 11 out of 13 schizophrenic cases studied at NIMH Neurosciences Center at St. Elizabeths and in no controls encourages us to continue. A replication is in progress.

Proposed Course of Project: Our immediate plans for this project are: a) to investigate further the cause of the cell transformation seen to date in the cells incubated with NIMH Neurosciences Center at St. Elizabeths' CSF, b) to identify the factor(s) responsible for cell transformation achieved with the St. Elizabeths' schizophrenia CSF, c) to repeat the culture and CSF experiments with a new group of patients and controls.

Tests planned for the coming year include the following:

1) Cloning, screening of clones, and sequencing of DNA isolated from transformed cell media.
2) Preparation of DNA probe to screen stored, transformed cells from previous cultures and schizophrenic and control CSF.

Publications:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02280-08 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Brain Tissue Transplantation in Primates

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

PI:	*R. J. Wyatt*	*Chief*	*NPB, NIMH*
Others:			
	W. J. Freed	*Section Chief*	*NPB, NIMH*
	M. J. Egan	*Sr. Staff Fellow*	*NPB, NIMH*
	A. Elkashef	*Commissioned Corp*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
Laboratory of Cerebral Metabolism, NIMH, Bethesda, Md. (D. Doudet)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues x(c) Neither

_(a1) Minors

_(a2) Interviews

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

To advance the work already performed in our laboratory with rats, adrenal medulla tissue was grafted to the denervated putamen of the rhesus monkey in our continuing research on brain tissue transplantation. Graft survival is erratic. In the most successful animal, the behavioral response produced by the graft has lasted one year. An instrument (the brain grafter) that facilitates grafting was developed and a patent has been awarded. Adrenal survival may be enhanced by the addition of nerve growth factor and other trophic factors.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: The objective of this program is to transfer knowledge gained from brain tissue grafts in the rodent into primates, and eventually into humans.

Methods Employed: These studies involve surgical, histological, and histochemical procedures in primates.

Major Findings: Considerable success has been achieved in grafting embryonic substantia nigra and young adult adrenal medulla tissue into rats to decrease rotational behavior produced by unilateral lesions of the substantia nigra.

Even though grafts have been introduced into humans, it is important to establish the animal procedures intermediate between rats and humans. For example, it is crucial to know whether grafts will survive and, if so, how well they survive in nonhuman primates. Our first series of rhesus macaque (Macaca mulatta) animals, using both adrenal medulla and rhesus embryonic substantia nigra, was, for the most part, unsuccessful. With one exception; grafts were not found.

A second series was slightly more positive; at least some tissue survived transplantation. Eight mature, adult male rhesus macaque animals received a unilateral neurotoxic lesion in the substantia nigra (including A10) region. At least 2 months later, each animal received a unilateral implant of either fetal rhesus substantia nigra tissue or tissue from its own adrenal medulla, and at least 2 months after implantation, each animal was killed for catecholamine fluorescence histochemistry. The first 2 animals (A1 and A2) received fetal substantia nigra tissue. The remaining animals (A3 through A7) received host adrenal medulla tissue.

For the embryonic substantia nigra implants, a rhesus monkey embryo (59 and 71 day) was surgically removed ex utero and placed in lactated Ringer's solution. The brain was dissected to obtain substantia nigra tissue in a manner analogous to the procedure for embryonic rat brain. The region of the midbrain that includes substantia nigra tissue was divided into pieces approximately 0.25 mm^3 in size. This tissue was drawn into a 22-gauge needle with an average of 6 pieces of tissue per injection in a volume of approximately 10 to 20 µl of Ringer's solution.

After removing bone and dura, the injection needle was lowered into the head of the caudate nucleus with stereotaxic coordinates corrected by X-ray determination of skeletal landmarks. The needle was lowered until it was within the caudate. The tissue was then injected and after 3 minutes, the needle was withdrawn.

For the 6 remaining animals (A3 through A8), the left adrenal was taken through a posterolateral retroperitoneal approach. A single longitudinal incision was made

through the adrenal capsule and cortex under a dissecting microscope. The adrenal cortex was peeled off and cortical fragments trimmed away. The adrenal medulla was divided into pieces of 0.25 mm^3. The tissue in the Ringer's solution was drawn into a 22-gauge needle with between 5 and 10 pieces of tissue in a volume of approximately 10 to 20 µl per injection.

In animal A3, stereotaxic placement of the adrenal graft was used as described above for the 2 animals implanted with fetal substantia nigra. Five injections were made into the caudate of animal A3. In the 5 remaining animals (A4 to A8), to provide more secure anatomic placement of grafts, the caudate was directly observed. With the aid of a surgical microscope, a window was cut through the body of the corpus callosum exposing the left lateral ventricle and caudate. A 22-gauge needle was inserted into the body of the caudate to inject the tissue.

Surviving graft tissue could be identified by the presence of specific catecholamine histofluorescence in the cell bodies of the implants. Neither animal implanted with fetal substantia nigra had any evidence of surviving catecholamine-containing graft tissue. In contrast, the 6 animals implanted with host adrenal medulla tissue had at least some surviving tissue in the parenchyma of the denervated caudate nucleus. The graft tissue itself appeared relatively healthy, although an accumulation of macrophages was noted adjacent to or surrounding some of the graft sites. The only damage to the host caudate associated with the implantation procedure was scar formation along the needle track.

Most graft sites were deep within the body of the caudate nucleus along the implant tract. Additionally, 2 graft sites were on the edge of the caudate nucleus. At least some parts of most grafts appeared to be fused with the brain parenchyma, but there was no evidence of caudate reinnervation. All graft sites demonstrated some diffusion of the catecholamines. Most fluorescent cells retained the typical, rounded appearance of adrenal chromaffin cells. A minority of cells developed polygonal shapes, and a few cells appeared to develop nerve-like fiber processes although these remained within the graft itself. A third series of animals has been more successful but results are preliminary.

In addition to grafting tissue directly into the striata of monkeys, tissue has been grafted into the frontal cortices. The advantage of grafting into the frontal cortex is that the surgery is considerably simpler than for grafts into the striatum, and it allows for developing surgical procedures that do not require lesioning animals or use of complex stereotaxic placement of grafts. Also, in some cases, animals do not need to be sacrificed to determine results. In this series of 6 animals, over 10,000 adrenal chromaffin cells survived.

An instrument has been built that allows us to carefully insert the grafts into the striatum using a stereotaxic instrument. This has increased graft survival in

monkeys and we are applying for a patent.

The instrument's main grafter consists of a series of cannulae designed to minimize tissue trauma and allow easy, precise placement within the brain. The principle involves the insertion of tissue housed in a protected enclosure; when the enclosure is withdrawn, the graft is left in place without additional pressure. The device has an outer guide cannula and 2 inserts. The first is an occluder used for initial penetration only, after which it is removed. The second insert's inner cannula is fitted with a stylet. The amount of space the tissue occupies, and therefore the size of the tissue, is determined by the spacing of the stylet in the cannula. Tissue is inserted into the tip of the inner cannula with the stylet fixed. The inner cannula is lowered, and the tissue left in the brain by lifting the inner cannula while the position of its stylet is fixed.

Stereotaxic Instrument: For use in Macaca mulatta, the instrument is designed to fit into a modified Kopf stereotaxic instrument (Model 1404, David Kopf Instruments, Tujunga, CA), although modifications can be made for use in other stereotaxic instruments. The stereotaxic frame assembly is equipped with 2 carriers (Kopf model 1460), a carrier on each frame bar.

Brain Grafter Construction: The brain grafter, of stainless steel or another rigid, sterilizable material, consists of 2 cannula assemblies, A and B. Cannula assembly A has an outside guide cannula and a stylet for making the initial penetration into the brain. Cannula A is affixed to a 10-mm long cuff or holder that fits over one end of the cannula. The tubing of cannula A extends 84 mm beyond its 10-mm cuff. The tubing has a .228-mm wall with an outer diameter of 1.65 mm and an inner diameter of 1.193 mm. Stylet A extends 95 mm beyond a holding knob. The knob has been trimmed on one side so that it can easily pass up and down as the 2 carriers vertically move past one another. Stylet A is brought to a point extending 1 mm beyond the outside of cannula A. A bevel on stylet A is tapered to be continuous with a similar bevel outside cannula A, allowing for smooth penetration into the brain.

Cannula assembly B consists of an outer cannula that extends 94 mm beyond its 10-mm cuff. Its inside diameter is .685 mm, the outside diameter is 1.066 mm, and it has a wall thickness of .177 mm. Its tip is beveled to give a cutting edge for punching tissue. Stylet B, with a diameter of .558 mm, extends 105 mm beyond its 10-mm long cuff.

Cannula assembly B fits into a holder assembly H, which consists of a hollow tube with a 1-mm wide viewing slot cut 1.5 cm lengthwise. On the viewing slot's edge are 1-mm marks for determining the distance between the cuffs of stylet B and cannula B. A set screw in H holds stylet B in a permanent mount in the barrel of the cannula assembly holder H. Thumb screw H on the cannula assembly holder H

maintains cannula B in a fixed position in relation to stylet B.

Use of Brain Grafter: During surgery, cannula assembly A with stylet A is stereotaxically lowered through a burr hole to a position where the graft is to rest. Stylet A_2 is removed. Cannula assembly B and holder assembly H are used to keep the stylet and the cannula at a fixed distance to allow the donor tissue to be punched and taken into the cannula. Using the millimeter markings on the view slot of holder H to determine the amount of tissue to be grafted, thumb screw H is tightened around cannula B. (For example, when the amount of tissue to fill the cannula is determined to be 2 mm, the 2 cuffs of the cannula assembly are placed 2 mm apart as determined by the view slot and markings.) Thumb screw H is tightened and the cannula assembly is used to punch the tissue to be grafted. After filling the cannula assembly B with the punched material, cannula assembly holder H is inserted into cannula assembly A. Stereotaxic carrier B is lowered onto assembly B and locked in by tightening its carrier screw. Thumb screw H is loosened, and cannula assembly A raised until contact is made with cuff B. As cannula B is raised, the tissue is dropped from the cannula and deposited in its proper place. Multiple injections can be made into the same track by simply raising cannula assembly A to the appropriate height and repeating the procedure.

Experience has taught us that cannula B's cross sectional sizes are the smallest that we can reliably use to punch adrenal medulla tissue from the monkey. Dimensions of the other cannula and stylets are determined by cannula B. Preliminary data indicate that the device may be superior to other techniques for transplantation of adrenal medulla tissue into the primate striatum. In a number of sites, tens of thousands of cells have survived; in other sites, only a few cells survive. Despite this inconsistency, the grafter gives better maximum survival of adrenal chromaffin cells than other techniques we have used in monkeys. The yield (survival of cells) using this device is also superior to what we have found in the parenchyma of the rat brain, where about 200 chromaffin cells per animal survive permanently when simply injected with a needle into the striatum. A U.S. patent has been awarded for this device.

Behavioral Response: Several animals have had multiple grafts placed into the right putamen after having MPTP lesions. The initial response of all animals has been to decrease the apomorphine-induced rotations away from the lesion. This rotation indicates the graft's success in that a failure to rotate after apomorphine suggests that dopamine has been replenished by the graft. One animal has had essentially a complete recovery following multiple adrenal grafts to the putamen. This success has continued for a year.

When the two animals which had adrenal grafts placed into the right side of their brain were examined histologically, it was learned that in both animals the grafts were slightly medial to the right putamen. In the animal whose turning was

stopped for a period of several months, but which returned to a pre-graft turning level, no evidence of graft survival could be found. In the animal whose turning remained suppressed throughout the year, a small number of cells were found in a bed at the end of the needle track, adjacent to, but not in the putamen.

A subsequent series of animals has been made using multiple injections of MPTP into the right internal carotid artery. These animals have stable turning. Prior to the insertion of the graft, these animals have had MRI head scans to insure better placement of the graft. In the one animal who has been sacrificed to date using stereotaxic placement following an MRI scan, the graft has still been slightly medial to the putamen. This animal had four tracts placed into its brain with small amounts of adrenal tissue placed at the bed of each tract. The grafts were sandwiched in Gel Foam impregnanted with nerve growth factor. In the two tracts where the nerve growth factor and Gel Foam were placed, small clusters between 50 and 75 fluorescent cells were found. These cells had characteristic neuronal shapes with processes extending from them. It is too early to determine whether the addition of the nerve growth factor increased the survival and formation of neuronal-like cells but in this preliminary study it appears to provide an advantage.

^{3}F-DOPA and Cerebral Blood Flow PET studies. Working with Drs. Doris Doudet and Robert M. Cohen (LCM, NIMH), we have found a large asymmetry in the striatum/cortex ratios and uptake rate constants between the MPTP-injected and non-injected sides of these hemi-parkinsonian animals. These PET studies were carried out up to 25 months after the last dose of MPTP, and demonstrated that the effects of this neurotoxin are lasting. We have also demonstrated, using a technique that reduces the background noise by about 50 percent, that there is reduced DOPA uptake in the striatum of the side not injected with MPTP, suggesting that MPTP is not completely trapped in the striatum during the first pass. In these studies, CSF homovanillic acid (HVA) and, to a lesser extent, CSF 5-hydroxyindoleacetic acid (5HIAA) were strongly correlated with ^{18}F-DOPA PET data. The lower 5-HIAA suggests that MPTP partially destroyed serotonin neurons in addition to those containing dopamine. Surprisingly, neither spontaneous nor apomorphine-induced turning correlated with ^{18}F-DOPA PET data or CSF HVA content. While there were no abnormalities in cerebral blood flow in these animals, the striatum on the lesioned side did not have low blood flow. On the unlesioned side, the blood flow was high, suggesting a compensatory mechanism was present.

Significance to Mental Health Research: These studies may lead to the development of tissue transplantation as a clinically therapeutic procedure for degenerative diseases and destructive lesions of the brain. Also, they may lead to an increased knowledge about development and regeneration in the brain in general. Since there is considerable evidence that some schizophrenic patients have altered brain structure (perhaps through degeneration, and degeneration is clearly involved in

diseases such as Alzheimer's), learning more about brain plasticity is of primary importance in understanding these illnesses.

Proposed Course of Project: Brain grafting should be seen as a potential treatment for disorders such as Parkinson's disease as well as a method for understanding plasticity in general. The course of this project should continue until such time as there is sufficient justification for bringing these techniques on a widespread basis into the clinic. At that time, further refinements and developments will probably be needed to maximize the potential benefits to patients. Because work with primates is inherently slow, progress will also be slow. Nevertheless, there do appear to be incremental enhancements in our ability to graft tissue in primates. Further work should probably await the development of viable engineered cells. Development of these cells is occurring in a number of laboratories including the Preclinical Neurosciences Section of our Branch. As soon as these cells have been demonstrated to work in rodents, we will use our experience with non-human primates to test the efficacy of these cells before they are introduced into patients.

Publications:

Doudet, D.J., McLellan, C.A., Aigner, T.G., Wyatt, R.J., Adams, H.R., Miyake, H. Finn, R.T., and Cohen, R.M.: Post-injection L-phenylalanine increases basal ganglia contrast in PET scans of 6-^{18}F-DOPA. Journal of Nuclear Medicine. 32:1408-1413, 1991.

Doudet, D.J., McLellan, C.A., Aigner, R.G., Wyatt, R.J., and Cohen, R.M. Improved evaluation of specific to nonspecific ^{18}F-dopa uptake in brain. Annals of Neurology, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02282-08 NPB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Neurovirology and Neuroimmunology of Schizophrenia

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

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	H. Kulaga	*Research Biologist*	*NPB, NIMH*
	D. Glovinsky	*Sr. Staff Fellow*	*NPB, NIMH*
	L. Wing	*IRTA Fellow*	*NPB, NIMH*

COOPERATING UNITS *(if any)*

Clinical Chemistry Service, NIH (N.M. Papadopoulos); NIAID, NIH (O. Preble); University of California at San Diego (S. Spector); Rocky Mountain Multiple Sclerosis Center (R. Murray); University of Colorado (S. Leonard)

LAB/BRANCH

Neuropsychiatry Branch

SECTION

Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

X(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 on neurovirology and neuroimmunology is focused on obtaining evidence that the pathogenesis of schizophrenia and other neuropsychiatric illnesses may involve an infectious process by a viral agent and/or an autoimmune reaction involving central nervous system tissue autoantibodies. The techniques used involve the quantification of specific proteins, including immunoglobulins, in the cerebrospinal fluid (CSF), stimulation of cultured lymphocytes in order to identify latent retroviral infection, the use of the polymerase chain reaction (PCR) in an effort to identify small quantities of viral genomic DNA in postmortem brain tissue, and the assessment of lymphocyte subpopulations.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: Despite a vast number of studies showing both structural and functional abnormalities in the central nervous system in schizophrenia, the etiology (or etiologies) of this disorder remains unclear. The goal of this project is to use newly developed techniques in molecular biology, virology, and immunology to study schizophrenia and other neuropsychiatric illnesses in search of a possible viral and/or autoimmune cause.

Methods Employed: CSF samples have been analyzed using rate nephelometry to measure both albumin and immunoglobulin G (IgG). If these components of CSF and serum are measured, one can estimate endogenous IgG production, a potential indicator of infection/autoimmunity in the central nervous system (CNS), while correcting for differences in blood-brain barrier permeability.

Plasma samples from schizophrenic patients on and off neuroleptic medications were analyzed by Dr. Preble for concentrations of alpha interferon, a general marker of immune activity.

A series of studies were done to test the hypothesis of retroviral involvement in schizophrenia. Lymphocytes were harvested from schizophrenic patients and control subjects and then established in tissue culture. Various induction methods were used with these cultured lymphocytes in an attempt to unmask any integrated retroviral DNA in the cells, including 5-azacytidine and gamma irradiation.

Another method employed in the project involves the use of the PCR technique, which can amplify extremely small quantities of viral DNA in tissue samples. This technique utilizes primer DNA sequences, specific to the virus of interest, to amplify the viral sequence if it is present in the tissue sample. This technique has been pursued on several fronts, looking for the presence of viral genomic DNA in brain tissue for three viruses potentially involved in neuropsychiatric disorders: human T-cell lymphotropic virus type I (HTLV-I), which has been implicated in multiple sclerosis (with Drs. Kulaga, Glovinsky, and Murray); and cytomegalovirus (with Drs. Alexander and Spector) and herpes simplex virus type I (HSV-I) (with Drs. Alexander and Murray), two viruses which have been implicated in schizophrenia.

Recently it has been reported that a specific lymphocyte cell type, the CD5 positive (CD5+) lymphocyte, which is associated with autoimmunity, may be increased in schizophrenic patients. Using specific monocloncal antibody

labeling and cell scanning techniques, the percentage of CD5+ cells in blood samples from schizophrenic patients with various neuropsychiatric disorders is being studied by Drs. Kulaga and Glovinsky and compared with normal control samples. Once isolated by cell sorting, these cells can be studied in order to determine their clonality and, ultimately, to assess the target of this immune activation.

Finally, we have pursued studies of brain tissue, seeking neuropathological abnormalities which might, in turn, reflect viral and/or autoimmune CNS damage.

Major Findings: In an initial study, a subset of patients with chronic schizophrenia was found to have increased central nervous system IgG production. In one patient there was evidence of oligoclonal banding when electrophoresis of CSF was performed. Expansion of this earlier study of CSF proteins yielded data (now in press) supporting the finding of increased CNS IgG production in a subset of patients, even when the calculation is corrected for increases in blood-brain barrier permeability (which also occurs in a subset of schizophrenic patients, allowing more peripheral protein to enter the CSF). This expanded cohort also allowed a within-subjects analysis on versus off neuroleptic medication showing that neither increased blood-brain barrier permeability nor elevated CNS IgG production appear to be related to neuroleptic treatment itself.

The data regarding plasma interferon concentrations indicated that, while there is an increased frequency of elevated interferon concentrations in patients with schizophrenia compared with control populations, these elevations were modest. Also, there was a trend toward increases in interferon being associated with neuroleptic treatment, as opposed to increases in interferon being associated with a neuroleptic-free state and increased psychosis.

Data obtained utilizing the PCR technique to amplify viral DNA sequences have been analyzed. Cytomegalovirus been proposed as an etiologic agent for schizophrenia. The PCR data regarding cytomegalovirus found no evidence in brain tissue from controls or schizophrenic subjects of residual viral DNA.

Three studies focusing on the use of lymphocyte cultures to test for evidence of retroviral infection by measuring reverse transcriptase activity have failed to show any evidence of the presence of a retrovirus in lymphocytes from schizophrenic patients. One study involved a standard culture technique; the second involved stimulation of cultures with 5-azacytidine, a technique that may unmask methylated sequences; and the third utilized exposure to gamma irradiation.

A neuropathological study looking for the presence of pathology in the basal forebrain nucleus basalis of Meynert has shown that schizophrenic patients, even those with prominent cognitive abnormalities, do not have the cell loss seen in this region in patients with other dementing neuropsychiatic disorders.

New Findings: Results (submitted for publication) using PCR to search for HSV-I in schizophrenia have been negative. Brain tissue from herpes encephalitis cases served as a positive control.

HTLV-I has been proposed as an etiologic agent in another neuropsychiatric disorder, multiple sclerosis. The current data from our PCR experiments seeking HTLV-I DNA in brain tissue from controls and patients with multiple sclerosis indicate that endogenous sequences with partial homology to HTLV-I and other retroviruses may be present in some cases from both groups. RNA studies show that these sequences may be expressed not only in human brain but also in other organs and species.

The work on gene expression raises the issue of the stability of mRNA in human postmortem brain tissue. In a collaborative study, Dr. Leonard demonstrated that mRNA usable for gene expression studies may be reliably isolated from human brain tissue with postmortem intervals up to 42 hours. It was found that long-term frozen storage may adversely affect the suitability of isolated RNA for studies requiring full-length transcripts.

Two papers are being prepared for publication regarding CD5+ lymphocytes in schizophrenia. An expanded cohort of schizophrenic patients has now undergone lymphocyte subset analyses, confirming reports by other groups of CD5+ lymphocyte increases associated with this diagnosis. Samples from patients with other psychiatric disorders, especially those with drug abuse, indicate that this finding is not specific to schizophrenia. Serial samples show the increase in schizophrenic patients to be stable over time. Work is in progress to examine the clonality of these lymphocytes. In addition, Dr. Wing recently completed an animal study showing that two drugs commonly used by schizophrenic patients, haloperidol and nicotine, may alter lymphocyte subsets.

Significance to Mental Health Research: Although studies in this area have yet to identify definitive evidence of viral (or retroviral) infection or autoimmunity in schizophrenia (or multiple sclerosis), the goals of the project remain important. Our data show abnormalities in CSF IgG and lymphocyte subsets

which indicate some immunologic mechanism in a subgroup of schizophrenic patients. If a viral infection and/or an autoimmune process are found to be involved in schizophrenia, this would provide an advanced understanding of the cause(s) of this disorder. The discovery of more effective treatments (or possibly the prevention) of schizophrenia is clearly dependent on better understanding of its etiology (or etiologies).

Proposed Course of Project: The projects involving use of the PCR technique to isolate viral genomic DNA in brain tissue from neuropsychiatric patients have required further experimental work in response to reviewers comments, but are now essentially complete and in varied stages of publication. Although the results are apparently negative in terms of directly implicating a virus in the disorders being studied, these are viewed as important because of the high level of sensitivity of the technique being employed and their utility in defining whether the CNS harbors latent viruses. The primary current focus is on better characterizing the CD5+ lymphocyte increases seen not only in schizophrenic patients but also other psychiatric diagnosis, including basic science studies to clarify the role of drug exposure on these lymphocyte alterations.

Publications:

Feenstra A, Kirch D, Coggiano M, Wyatt R. New experimental approaches examining a viral etiology of schizophrenia. In: Kurstak E, ed. Psychiatry and Biological Factors. New York: Plenum, 1991;149-58.

Kirch D, Wyatt R: Interferon and immunoglobulin G as immunologic markers in chronic schizophrenia. In: Kurstak E, ed. Psychiatry and Biological Factors. New York: Plenum, 1991;197-207.

Kirch D, Wagman A, Goldman-Rakic P. Commentary: The acquisition and use of human brain tissue in neuropsychiatric research, Schizophrenia Bulletin 1991;17:593-96.

El-Mallakh R, Kirch D, Shelton R, Fan K-J, Pezeshkpour G, Kanhouwa S, Wyatt R, Kleinman J. The nucleus basalis of Meynert, senile plaques, and intellectual impairment in schizophrenia, Journal of Neuropsychiatry and Clinical Neurosciences, 1991;3:383-86.

Coggiano M, Alexander R, Kirch D, Wyatt R, Kulaga H. The continued search for evidence of retroviral infection in schizophrenic patients, Schizophrenia Research, 1991;5:243-47.

Kirch D, Alexander R. Viruses, autoimmunity, and psychiatric disorders. In: Tasman A, Riba M, eds. Annual Review of Psychiatry, Vol. 11. Washington, D.C.: American Psychiatric Press, 1992;202-18.

Alexander R, Spector S, Casanova M, Kleinman J, Wyatt R, Kirch D. Search for cytomegalovirus in the postmortem brains of schizophrenic patients using the polymerase chain reaction, Archives of General Psychiatry, 1992;49:47-53.

Kirch D, Alexander R, Suddath R, Papadopoulos N, Kaufmann C, Daniel D, Wyatt R. Blood-CSF barrier permeability and central nervous system immunoglobulin G in schizophrenia, Journal of Neural Transmission, in press.

Leonard S, Logel J, Luthman D, Casanova M, Kirch D, Freedman R. Isolation and quantification of RNA from human postmortem brain collections, Brain Research, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02373-06 NPB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

The Effects of Cocaine on Central and Peripheral Catecholamines

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

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LAB/BRANCH

Neuropsychiatry Branch

SECTION

Clinical Neuropsychiatry

INSTITUTE AND LOCATION

NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  _(a2) Interviews

_(b) Human tissues

x(c) Neither

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

That stimulants can be associated with a paranoid psychosis in otherwise normal individuals is frequently offered as evidence for the involvement of the dopamine (DA) system in psychotic illness. One animal paradigm designed to study this phenomenon has been the chronic administration of amphetamines (AMPH), agents known to enhance release and block uptake of DA. In numerous animal studies, infusion of relatively low doses of meth-AMPH has been associated with the depletion of DA and its metabolites, a decrease in tyrosine hydroxylase, a decline in DA receptors, and the structural changes consistent with neurotoxicity. These changes have been found most frequently in the striatum, olfactory tubercle, and cortex. To further examine this mechanism, we chose to study the biochemical effects of cocaine, a stimulant known to inhibit the uptake of central catecholamines. We chronically administered cocaine (10 mg/kg) or saline twice daily to rats by intraperitoneal injection for 1, 2, and 3 weeks. In a series of experiments, the frontal cortex, nucleus accumbens, caudate nucleus, and hypothalamus were removed at intervals ranging from 1 hour to 3 months after the last dose. Norepinephrine, DA, and their metabolites were measured by mass-fragmentography. We found a persistent, significant reduction in dopamine and in the dopamine metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in the frontal cortex and hypothalamus, an observation lending further evidence to the links between stimulants and persistent dopamine deficit states.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: The primary objective of this investigation is to assess the suitability of using rats chronically treated with cocaine as an animal model of schizophrenia. We intend to further characterize the effects of chronic cocaine administration in the rat on the turnover of biogenic amines, on receptor physiology, and on indicators of neuronal toxicity, including immunohistochemical staining and quantitation of neuropeptides. We also intend to study possible mechanisms which could produce alterations in dopaminergic neurons. Finally, we will look at interactions of other pharmacological and non-pharmacological interventions clinically relevant to schizophrenia and other psychotic illnesses with this paradigm.

Methods Employed: All biochemical assays of biogenic amines and their metabolites will be performed by combined gas chromatographic mass spectrometry or high pressure liquid chromatography. We also intend to explore the use of combined high pressure liquid chromatography and mass spectrometry (LC-MS) for the identification and quantitation of metabolites. Immunohistochemical studies will be done and will involve staining of tyrosine hydroxylase and dopamine beta hydroxylase-positive fibers in the frontal cortex and striatum. Receptor physiological studies will also be done.

Major Findings:

1. Chronic cocaine administration (10 mg/kg) for periods ranging from 1 to 3 weeks significantly reduced dopamine turnover in the frontal cortex, caudate, septum, and nucleus accumbens during treatment.

2. One week after cessation of cocaine administration, dopamine turnover had normalized in all brain regions measured except the frontal cortex and hypothalamus.

3. To further assess the effects of chronic cocaine on central dopamine metabolism, we administered cocaine (10 mg/kg) to rats for 1 week, and measured frontal, cortical and hypothalamic dopamine and its metabolites 6 weeks and 3 months after termination of administration. In the frontal cortex, combined molecular concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) remained significantly reduced at 6 weeks and 3 months. In the hypothalamus, DOPAC + HVA was significantly reduced only at the 6-week point.

4. Our results indicate a persistent reduction of dopamine turnover in the frontal cortex and in the hypothalamus following 1 week's administration of cocaine.

5. We have studied the effects of chronic cocaine administration on neuropeptides thought to be co-localized in dopamine terminals. Thus far we have found a reduction of neurotension in the nucleus accumbens following 24 hours and 3 weeks withdrawal from cocaine. Cholecystokinin was reduced in the frontal cortex, nucleus accumbens, and caudate following 3 weeks withdrawal from cocaine.

6. We have studied the effects of chronic cocaine administration during increasing periods of withdrawal, ranging from 1 hour to 6 weeks, on the dopamine transporter as reflected in the binding of the compound GBR. We have found evidence suggesting that a persistent reduction occurs in these binding sites, consistent with the hypothesis that cocaine exerts neurotoxic effects on dopamine neurons.

7. Since dopamine is known to modulate the release of prolactin, we have measured blood prolactin in rats during various stages of withdrawal from cocaine. Thus far we have found persistent effects in blood prolactin up to 3 weeks after withdrawal from cocaine.

8. By staining dopamine neurons in the frontal cortex, striatum, and substantia nigra with an antibody to tyrosine hydroxylase, we have found preliminary evidence that in some animals, observable destruction of dopamine neurons occurs. The evidence from the measurement of dopamine metabolites, dopamine uptake receptor sites, neuropeptides co-localized with dopamine, and histochemical studies supports the hypothesis that chronic cocaine produces neurotoxic effects on dopamine neurons.

9. One possible mechanism which could produce alterations in dopaminergic projections is through the production of and subsequent neurotoxic actions of 6-hydroxydopamine. This compound has been implicated in the neurotoxic effects of amphetamine. Using GCMS, we have determined that cocaine does not produce measureable levels of 6-hydroxydopamine. Furthermore, previous reports of increased 6-hydroxydopamine levels after amphetamine may have mistakenly identified a chemical agent with a similar retention time on HPLC.

Significance to Mental Health: Our findings are consistent with clinical symptoms and neurochemical abnormalities observed in chronic cocaine abusers. Both cocaine abuse and disturbances in central dopamine have been associated with each of the following: both acute and chronic psychosis, euphoria, anhedonia, mood disturbance, and deficit states (flatness of affect, loss of motivation, impairment of attention and concentration). Cocaine abuse has also been associated with an increase in serum prolactin - a finding consistent with a dopamine depletion state. The finding of persistent reduction in central and periphernal dopamine metabolites in this model provides a possible biochemical mechanism for the

clinical symptoms observed in cocaine abusers. It suggests that subjects withdrawing from cocaine should be followed for evidence of peripheral and central dopamine depletion. It also suggests possible therapeutic interventions that may reduce the incidence of relapse in these patients. This finding also provides further evidence for the involvement of the dopamine neurotransmitter system in the deficit, or "negative symptom," state frequently observed in patients with chronic schizophrenia.

Proposed Course of Project: We plan to further characterize the effects of chronic cocaine administration on biogenic amine metabolites, serotonin and the possible increase in neurotoxic processes, such as those mediated by free radicals and glutamate. We also plan to further study the time course of the effects discussed above and the effects of multiple exposures (simulating cocaine binges) on biochemical measures, receptor physiology, and measures of neurotoxicity. We will investigate both pharmacologic agents (e.g., neuroleptics, free radical scavengers, and glutamate antagonists) and nonpharmacologic interventions (stress paradigms) on this model.

Publications:

Wehlestedt C, Karoum F, Jaskiw G, Wyatt RJ, Larhammar D, Ekman R, Reis DJ. Cocaine-induced reduction of brain neuropeptide Y synthesis dependent on medical prefrontal cortex, Neurobiology 1991;88:2078-2082.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02420-05 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effect of Chronic Exposure to Cocaine on Metabolism of Catecholamines

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

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COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Molecular Neuropsychiatry

INSTITUTE AND LOCATION
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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.4	0.1

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  _(a2) Interviews

_(b) Human tissues

X(c) Neither

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

Cocaine is a habit-forming stimulant that produces "reinforcing" effects. Central dopamine has been hypothesized to play a major role in the expression of pharmacological effects of cocaine, but direct biochemical support for this view is sparse and inconsistent. The latter may be due to two important factors: Most information on the biochemical effects of cocaine has come from acute studies, and the striatum and nucleus accumbens have been the brain regions most frequently analyzed. Therefore, certain important brain regions may have been overlooked. We have systematically evaluated the effects of 1 to 3 weeks chronic cocaine administration on central and peripheral biogenic amines, in particular, the catecholamines. Results revealed a preferential, long-term reduction of dopamine turnover and/or metabolism in both the periphery and the brain. The frontal cortex and the hypothalamus apparently are the areas most affected.

We have concentrated our efforts on understanding the mechanism by which cocaine reduces frontal cortex DA production and metabolism. A careful search for 6-hydroxydopamine (6-OHDA) in the frontal cortex and striatum with gas chromatography/mass spectrometry revealed no endogenous 6-OHDA in these brain regions. Administration of high doses of cocaine, methamphetamine and dopamine-releasing agents failed to produce detectable concentrations of 6-OHDA in the brain. A substance with a retention time close to 6-OHDA was occasionally detected, and may be the substance found by HPLC in the striata of rats given high doses of amphetamine. We hope to identify this substance and follow its origin and metabolic fate by using a triple quadrupole mass spectrometer.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: The primary objective is to assess the suitability of using rats chronically treated with cocaine as an animal model of schizophrenia. To accomplish this, the behavioral and biochemical effects of cocaine must be characterized, and the results compared with the various clinical manifestations of schizophrenia. An immediate objective is to first evaluate the effects of chronic cocaine exposure on central and peripheral biogenic amines (in particular, the catecholamines), serotonin, and other neurotransmitters. Once we have successfully completed this investigation, we will assess the kinetics of cocaine metabolites and their transport from the periphery into the brain, and determine how certain antipsychotic drugs interact with cocaine, both behaviorally and biochemically.

Methods Employed: All biochemical assays of biogenic amines and their metabolites will be performed by combined gas chromotography/mass spectrometry. These methods were developed in this laboratory and have been extensively applied to a wide range of clinical and basic science projects.

A number of highly sensitive procedures employing combined capillary column gas chromatography/positive and negative chemical ionization mass spectrometry were used to assay 6-ODHA in the brain.

Major Findings: We have evaluated the effects of 1-, 2-, and 3-weeks' repeated-biogenic amines. Peripheral biogenic amines were assessed by following their rate of excretion. In the brain, catecholamines and their metabolites were measured in the various brain regions (the hypothalamus, frontal cortex, septum, nucleus accumbens, striatum, and hippocampus) that are expected to play some role in the expression of cocaine's central effects.

Chronic cocaine exposure produced a preferential, long-term reduction in dopamine and phenylethylamine turnover and/or metabolism. This effect was detected 6 weeks after termination of 7 days' exposure to cocaine. This result suggests a long-term down regulation of peripheral dopamine turnover, a phenomenon similar to that observed in chronic schizophrenia (Arch Gen Psych 1987;44:604-7).

While acute cocaine treatment (10 mg/kg) was ineffective, chronic exposure to cocaine for periods ranging from 1 to 3 weeks significantly reduced dopamine turnover in the frontal cortex, striatum, septum, and nucleus accumbens. One week after termination of treatment, dopamine turnover, as assessed by its rate of metabolism, was normal in all regions analyzed except in the frontal cortex and hypothalamus.

To assess the long-term effects of cocaine exposure on central dopamine, we exposed rats to one week of cocaine treatment, and measured hypothalamic and frontal cortex dopamine and its metabolites 6 weeks and 3 months after termination of

cocaine treatment. The combined molecular concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) remained significantly low in the frontal cortex at 6 weeks and at 3 months. In the hypothalamus, DOPAC + HVA was low only at the 6-week point.

The results of our studies so far suggest a long-term effect of cocaine exposure on dopamine turnover in the hypothalamus and the frontal cortex. Some of the impairments observed in cocaine abusers can be attributed to disturbances in frontal cortex and hypothalamic functions. These include cognition, reward, emotional instability, and disturbances in hormone secretions regulated by the hypothalamic-pituitary-adrenal axis.

Dynamic measures of dopamine release and turnover in four brain regions of rats administered cocaine repeatedly for one week, and killed one week after withdrawal, showed clear regional differences. Findings may explain some of the short- and long-term biochemical and behavioral changes induced by repeated cocaine intake. In the frontal cortex and hypothalamus, dopamine turnover was reduced, while in the nucleus accumbens and striatum, dopamine turnover, one week after cocaine withdrawal, was stimulated. 3-Methoxytyramine (3MT) rate of formation, a good index of dopamine release, was increased only in the frontal cortex. These results indicate that chronic exposure to cocaine down-regulates dopamine turnover in the frontal cortex and hypothalamus, and up-regulates turnover in the nucleus accumbens. Dopamine release, on the other hand, appears to remain normal in all four regions except in the frontal cortex where the increase in 3MT formation may be the result of reduced dopamine uptake rather than increased release.

Significance to Mental Health Research: The successful development of an animal model of schizophrenia in itself will be of great scientific value. It will allow the convenient testing of new antipsychotic drugs and will offer better insight into the role of various neurotransmitters in schizophrenia.

Achieving our final goal - the development of an animal model of schizophrenia - will be accompanied by a new understanding of cocaine's pharmacology and its effects on central amines. These observations will be valuable in aiding future strategies in the treatment of cocaine addiction.

Proposed Course of the Project: To fulfill most of our future objectives, new methodologies will need to be explored. This will involve the use of combined high pressure liquid chromatography and/or super critical fluid chromatography (SFC) and mass spectrometry (LC-MS) as well as tendum mass spectrometry (MS-MS) for the identification and quantitation of metabolic products of drugs. Since MS-MS is a technique that merits exploration in the distant future, we plan to concentrate most of our near-term research efforts on exploring LC-MS techniques which are ideally suited to the identification of the 6-ODHA-like substance that was detected in the brain at high concentrations after cocaine was administered.

LC-MS and SFC are novel analytical tools that have not been fully explored in neuropharmacology. They are ideally suited to investigations aimed at monitoring the bioavailability of drugs. Furthermore, due to the rapid advances in high pressure liquid chromatography (HPLC) in recent years, it is now possible to custom design HPLC columns that offer separation powers and efficiencies that far exceed the performance of gas chromatography. LC-MS is expected to be extensively employed in the rapid and accurate measurements of drugs in various biological media. Another important application of LC-MS will undoubtedly be in the screening for the presence of drugs of abuse in urine and blood. SFC is potentially a very powerful technique that has not been fully explored.

Since we do not have the appropriate instrument (Triple quadrupole mass spectrometer) to identify the 6-ODHA-like substance found in the brain, we are currently employing a number of biochemical and pharmacological approaches to gain as much knowledge as possible of its chemical properties.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02428-05 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biological Properties of Intraventricular Grafts

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

PI:	*M.Poltorak*	*Visiting Scientist*	*NPB, NIMH*
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	A. Sanchez	*Visiting Associate*	*NPB, NIMH*
	M. Truckenmiller	*Sr. Staff Fellow*	*NPB, NIMH*
	H. Kulaga	*Research Biologist*	*NPB, NIMH*
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COOPERATING UNITS *(if any)*

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INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews

x(b) Human tissues

_(c) Neither

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

We have been studying selected immunohistological staining patterns of intraventricular tissue grafts in host brains and adrenal medullae in situ and in vitro. We have attempted to determine the essential factors involved in the development and survival of these tissues. Results indicate that the expression of cell adhesion molecules (CAMs) in transplanted cerebella is relatively undisturbed, and that genetically normal cerebellar grafts can survive in a mutant host environment defective in CAMs and myelination. We have also found that intraventricular grafts, as compared to intraparenchymal grafts, are not as well integrated with host brains, and that CAMs are present in rat adrenal medullae in situ. In tissue cultures, the outgrowth of fibers on chromaffin cells is associated with an enhancement of L1 and N-CAM on their neurites. Transplanted, surviving adrenal medulla fragments within the lateral ventricle of the host rat demonstrated an enhancement of L1 expression which was accompanied by a reorganization of the closely associated extracellular matrix. Other data suggest that only in xenografts does an intense host reaction occur that is capable of destroying transplanted tissue. MHC class II immunoreactivity is enhanced in host parenchyma on microglial cells, but not on GFAP positive astrocytes after iso, allo and xenografting. Genetic disparity does not seem to be the only factor affecting the survival of allogenic neural grafts, with or without systemic sensitization, and, in fact, plays only a relatively limited role. Susceptibility to neural graft rejection may depend on immunogenicity of the donor, Ir gene, host allele, and host susceptibility to autoimmune disease.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: Intracerebral grafting has already been used in humans. It has become important to clearly determine the properties and possibilities of intracerebral grafts. The cerebellum is a useful tissue for studies of nervous system development since it contains a limited number of cell populations arranged in a stable, trilaminar fashion with very restricted connections. It has been demonstrated that cerebellar grafts develop relatively normally in host tissue, despite the absence of external inputs. These studies were designed to determine what factors are essential for completed cerebellar graft development, and to demonstrate the immunocytochemical patterns of several antigens involved in the histogenesis of cerebellar tissue after transplantation. We also wanted to shed some light on the immunological features occurring in host brains after allo and xenotransplantations as compared to isografts. In addition, we have studied the effect of blocking Ia antigens within the host brain on the survival of intraventricular xenografts. We have also sought to determine whether the induction-rejection response against the established allografts in host rat brains is irrevocable after sensitization with donor tissue; in other words, whether certain rat strain combinations are resistant to brain graft rejection.

Since the CAMs are involved in the regeneration and survival of neural tissue, it seemed important to determine the expression of CAMs from the L2/HNK-1 family in adrenal medullae in situ, as well as in cultured chromaffin cells with and without NGF stimulation, and to draw a comparison with CAM expression after intraventricular transplantation. We also studied the neurite outgrowth of mesencephalic neurons on the purified CAMs and their fragments in short term cultures. These observations would be useful for understanding the role of CAMs in survival and mechanisms of intraventricular adrenal medulla and substantia nigra grafts.

Methods Employed: Intracerebral transplantations were performed in mice and rats. The embryonic donor tissue or adrenal medulla fragments from young adult rats was stereotaxically implanted into the lateral ventricle or into brain parenchyma. Some rats received mini-osmotic pumps filled with antibodies or vehicle. Additionally, host rats were systematically sensitized with donor tissue. After transplantation, the animal brains were sectioned and stained by tinctorial and immunocytochemical methods with primary antibodies against phosphorylated and nonphosphorylated neurofilament epitopes, myelin associated glycoprotein, myelin basic protein, glial fibrillary acid protein, neural cell adhesion molecules, L1 molecules, J1/tenascin molecules, L_2/HNK-1 epitopes, MHC class II molecules and helper T cell determinants. The peroxidase-antiperoxidase and indirect immunofluorescence methods were used. Adrenal gland sections and cultures of

One possible application of HCN-1 cells includes intracerebral transplantation. We also examined the survival of dissociated HCN-1 cells implanted into rat brain parenchyma. The host animals were not immunosuppressed. Despite expression of MHC class I antigens, small clusters of HCN-1 cells survived in the rat brain. These xenografts did not induce distinct immunological responses within the host brain tissue. Surviving HCN-1 cells demonstrated similar features to those observed in culture.

Our preliminary results suggest that the HCN-1 cell line would be suitable for intracerebral transplantation in primates or humans. However, it may be that short-term host immunosuppression or addition of HCN-1 cell differentiation factors would be beneficial for enhanced cell survival.

(ii) The A7 cell line is an astrocyte-like cell immortalized by SV40 large T antigen, using retroviral-mediated gene transfer. These cells were transplanted into rat brains, and the graft-host interaction was investigated immunohistochemically. The A7 cells survived focally 2, 6 and 8 weeks after transplanation, and retained the immunocytochemical properties observed *in vitro*. No immunological response was observed. GAP-43 and N-cadherin immunoreactivities were not expressed by A7 cells, but were seen in the matrix within the area of the graft and in the surrounding brain tissue. This indicates that A7 cells may stimulate expression of GAP-43 and N-cadherin immunoreactivity by host tissue. Expression of Thy 1.1 was not observed within the graft site after 2 weeks of survival, but, 6 and 8 weeks after transplantation, Thy 1.1 was observed within the graft area, indicating the possible co-existence of grafted cells and host tissue. Although indirect, these observations suggest that the A7 cells induce changes in the host brain, including possible growth or regeneration of host tissue into the graft area.

(iii) We have evaluated neurite outgrowth from mesencephalic tyrosine hydroxylase-positive neurons grown in vitro on different substrates. Cultures of ventral mesencephalon from rat embryos (E13) were plated on plastic dishes coated with the following substrates: L1, L2/HNK-1"residual" (mainly J1/160 but also tenascin), and MAG antigens from mouse brains, laminin, fibronectin, poly-L-lysine, RGD peptide and plastic alone. After 3, 4 and 6 days in vitro, the cultures were stained using an antibody against tyrosine hydroxylase (TH), and the length of TH-positive neurites was measured by computer assisted image analysis in a double blind fashion. L1 antigen had a significant positive effect on neurite outgrowth as compared to the other substrates studied. Laminin and fibronectin were also favorable substrates. In cultures treated with cytosine arabinoside to prevent mitosis and glial proliferation, the positive effect of L1 was abolished, but laminin still had a stimulatory effect. These data indicate that L1 may be indirectly involved in differentiation or axonal elongation of substantia nigra dopaminergic neurons, and suggest a complex effect involving both neurons and glia on dopaminergic neurite development.

(iv) Brain injury induces trophic effects within adjacent tissue through an unknown molecular mechanism. One model of this lesion effect involves the enhanced outgrowth of neuronal processes from transplanted substantia nigra (SN) in animals with cerebral cortex lesions. Since cell adhesion molecules (CAMs) are involved in the molecular mechanisms of contact between cells and surrounding extracellular matrix components, and are important in plasticity of the nervous system, we investigated changes in the CAMs (L1, N-CAM and tenascin) as well as synapse-associated proteins and gliosis in the striatum of mice with cortical lesions. Lesioned mice showed a significant enhancement of both L1 and N-CAM immunostaining intensity within the most medial-periventricular and dorso-medial parts of the striatum, as compared to the non-lesioned side. Tenascin expression was significantly decreased, but only in the most medial part of the striatum on the lesioned side. Intensity of immunostaining with L1, N-CAM and tenascin did not show significant changes related to time after lesioning. These changes were accompained by decrease of synapsin and synaptophysin expression in the most medial part of striatum. GFAP immunoreactivity was also increased in the dorsal and, especially, in the dorso-lateral quadrants of the striatum on the lesioned side. These changes in CAM expression indicate a possible molecular basis of lesion-induced plasticity in neuronal circuits within the striatum.

(v) We studied immunological reactions after intraventricular iso-, allo- and xenogenic transplantation of adrenal medulla. We found that in xenografts only, T cell infiltration and graft rejection were observed. Isografts and allografts were not rejected, although expression of MHC class II antigen was observed at all survival times. Although T-cell mediated rejection was found for xenografts only, the survival of chromaffin cells in allografts was decreased as compared to isografts. Although survival of intraventricular adrenal medulla allografts is limited, it appears that this limited survival is not due to T cell-mediated graft rejection.

(vi) To investigate the factors related to allogenic brain graft rejection, we have transplanted fetal rat cortex from either LEW-RT1l or BN-RT1n rats into brains of several isogenic and allogenic inbred strains: F344-RT1l, LEW-RT1l, BN-RT1n, AO-RT1u, PVG-RT1c, PVG-RT1u and PVG-RT1l . Each donor and host combination was subsequently divided into two subgroups. One group was systematically sensitized twice with donor skin tissue and another group received sham sensitization procedures. Four weeks after the second sensitization, the host brains were investigated histologically and the percentage volume of each graft that showed increased cellularity was estimated. Immunoreactivity for MHC antigens and T cell antigens was also studied immunocytochemically. In some animals, to examine systemic immune reaction, blood samples from the inferior orbital sinus, deep cervical lymph nodes, or spleen were investigated by flow cytometric analysis. Almost all grafts in sham sensitized animals from all groups survived well. Under these conditions, immunological rejection responses were usually minimal, but two strains (AO-RT1u and F344-RT1l) showed considerable cell infiltration and

expression of MHC antigens even without sensitization. After sensitization, all allogenic strain combinations showed greater signs of rejection responses. The severity of the rejection responses, however, varied considerably between groups. All the grafts from BN-RT1n donors were rejected severely in all host strains. For LEW-RT1l donors, grafts survived well in some host strains (BN-RT1n, AO-RT1u, PVG-RT1c, PVG-RT1u) even with complete genetic disparity. Curiously, F344-RT1l hosts rejected LEW-RT1l grafts even though the two strains have the same main MHC-loci, but different non-MHC loci. For both donor strains, EAE-susceptible hosts showed relatively large rejection responses. Histological data and flow cytometric analysis indicated that CD4+ cells were predominant in allograft rejection responses. Our results suggest that genetic disparity is not the only factor affecting the survival of allogenic neural grafts, with or without systemic sensitization, and, in fact, plays only a relatively limited role. It is suggested that susceptibility to neural graft rejection depends on a number of factors, most imporantly immunogenecity of the donor, but also including Ir gene, host MHC allele, and host susceptibility to EAE or autoimmune disease.

Proposed Course of Project.

(i) We have shown that the L1 substrate promotes neurite outgrowth of tyrosine hydroxylase-positive mesencephalic neurons in culture. We would like to test whether the protease degradation fragments of L1 antigen have similar properties. This is a potentially important issue since it is possible that the mechanisms of action of neuronal tissue or adrenal medulla grafts may consist of supplying CAMs or their fragments to the host brain.

(ii) The CAMs are involved in the regeneration and development of the nervous system. Although schizophrenia is a disorder of unknown origin, some data suggest involvement of possible neuronal developmental disturbances. Moreover, it has been claimed that the serum of schizophrenic patients contain 70 kD fragments of N-CAM. We would like to test the biological fluids of schizophrenic patients for presence of CAMs.

(iii) We have shown that the cortical frontal lesions induce enhancement of L1 and N-CAM immunoreactivity and simultaneously decrease tenascin expression in the medio-dorsal striatum on the lesioned side as compared to the medio-dorsal striatum on the nonlesioned side. We would like to test, in similar experiments, other CAMs immunoreactivities as well as to include additionally the influence of the substantia nigra lesion on the striatal CAMs expression.

(iv) Since the CAMs molecules are involved in neurite outgrowth, we would like to attempt to test whether direct injection into the brain of CAMs or their fragments has any effect on regeneration capacity of the central nervous system. As a long-term goal, we would like to eventually introduce the CAMs genes into the

neuronal cells. Ultimately, these methods could be applied to influence organization and regeneration of striatal ciruits.

(v) We have shown that the sensitization-induced allograft rejections is a complex phenomenon, and, for some rat strain combinations, graft rejection did not occur despite complete genetic disparity. The immunological mechanisms underlying these events are unknown. We would like to evaluate these reactions using PCR methods.

Significance to Mental Health Research: Intracerebral grafting has already been performed successfully on humans in the treatment of Parkinson's disease. Although the current application of neural grafting is confined to this disorder, with one known cell deficit, it is possible that techniques related to neural grafting can eventually be applied to other disorders. This new approach requires further basic studies to determine the properties and possibilities of grafting.

Publications:

Poltorak M, Freed WJ. BN rats do not reject F344 brain grafts even after systemic sensitization, Ann Neurol 1991;29:377-388.

Poltorak M, Isono M, Freed WJ, Ronett GV, Snyder SH. Human cortical neuronal cell line (HCN-1): Further in vitro characterization and suitability for brain transplantation. Cell Transplantation 1992; 1: 3-15.

Poltorak M, Shimoda K, Freed WJ. L1 substrate enhances outgrowth of tyrosine hydroxylase immunoreactive neurites in mesencephalic cell culture. Exp Neurol, in press.

Isono M, Geller HM, Poltorak M, Freed WJ. Intracerebral transplantation of the A7 immortalized astrocytic cell line. Restorative Neurology and Neuroscience, in press.

(4) We have also studied the expression of glial fibrillary acid protein (GFAP) in intraventricular mouse cerebellar grafts. We were unable to find the characteristic, GFAP-positive, elongated, radial processes of Bergmann glia in the grafted tissue. Since normal granule cell migration was observed in grafts, it is possible that non-radially oriented GFAP-positive glia or GFAP-negative radial glia may permit migration of granule neurons in transplanted tissue.

(5) We have studied the histochemical features of immunological reactions to intraventricular allografts and xenografts and have made comparisons with those of isografts. Xenografts provoked an intense immunological reaction involving MHC class II immunoreactive cells and helper T cells. The results suggest that the process of tissue implantation and its associated brain injury induces enhanced MHC class II immunoreactivity on microglial cells but not on GFAP-positive astrocytes, within and surrounding iso, allo and xenografts. Despite this predisposition to immunological reactions, only in xenografts did the reaction proceed through all of the steps required for a graft rejection response, ultimately leading to the destruction of the grafts.

(6) We have studied the influence of chronic infusions of antibodies into the CSF against MHC class II antigen on immunological reactions to intraventricular xenografts in rats. The chronic infusion of antibodies with osmotic pumps implanted one day before grafting did not prolong xenograft survival. Furthermore, we have observed intense granulomatosis reactions in the host brains of animals infused with antibodies against Ia antigen and with control antibodies against penicillin. These reactions were not observed in the animals that received antibody infusions without xenografts. It appears that this reaction was produced by the massive and persistent injection of heterologous immunoglobulins, together with the presence of xenograft tissue.

(7) We have studied the expression of a series of cell adhesion molecules (CAMs) (L1, N-CAM, J1/tenascin molecules and MAG and their common L2/HNK-1 epitopes) in normal rat adrenal gland sections, as well as in adrenal medulla cell cultures, with and without NGF stimulation. In situ L1 and N-CAM immunoreactivity was present on chromaffin cells and in surrounding connective tissue. The extracellular matrix of whole medullae also expressed J1 molecules. In long term cultures, NGF stimulation enhanced both L1 and Thy 1.1 immunolabeling on chromaffin cells and their processes. NGF-activated chromaffin cells also demonstrated neurofilament and vimentin-like immunoreactive filaments within cell bodies and their processes. Chromaffin cells were usually found on a layer of N-CAM and fibronectin-positive fibroblasts, and often were associated with laminin immunoreactive material. These data suggest a possible role of N-CAM and L1, as well as ECM laminin process outgrowth from chromaffin cells.

(8) We have also studied the expression of CAMs in adrenal medulla fragments implanted into the lateral ventricle. Surviving implanted chromaffin cells showed enhancement of surface L1 expression as compared to normal chromaffin cells in adrenal medullae. The implanted chromaffin cells demonstrated only a partial conversion to neuronal phenotypes. Surviving chromaffin cells were accompanied by a reorganization of their closely associated extracellular matrix (ECM). As compared to normal in situ adrenal medullae, graft ECM demonstrated a substantial increase in L1 and laminin immunoreactivity and a decrease in J1/tenascin expression. Some adrenal medulla grafts degenerated, and, in these cases, grafts showed a fragmentation of ECM and a gradual disappearance of CAMs. These results suggest that surviving adrenal medulla grafts exhibit an increased synthesis of certain CAMs by chromaffin cells which may be involved in the interaction between chromaffin cells and the surrounding ECM. It is speculated that both surviving and degenerating adrenal medulla grafts could provide CAM and ECM components, including laminin, to host brains, and, in this way, contribute to the functional effects of grafts.

(9) It is generally believed that brain tissue allografts will not survive it the host animal is systemically sensitized, by skin grafting or other means, to MHC antigens of the donor animal. We found that Fisher 344 brain grafts survive in Brown-Norway hosts even when the host is systemically sensitized to Fisher 344 tissue. Moreover, we observed extensive enhancement of MHC class I immunoreactivity in parts of grafted tissue developing within the third ventricle, but not for the same type of graft in the lateral ventricle. The MHC class I enhancement was not accompanied by substantial infiltrations of cytotoxic lymphocytes. Our findings thus suggest that neural graft rejection depends on general genetic susceptibility to immune reactions, and not only on disparity between donor and host antigens encoded by the MHC. Moreover, enhancement of MHC class I and class II expression within transplanted tissue does not predict graft rejection.

Major New Findings:

(i) The human neuronal cell-1 (HCN-1) line has recently been established. Under favorable conditions, these cells differentiate into mature neuronal phenotypes. We found that cultured HCN-1 cells express fibronectin immunoreactivity and grow well on fibronectin substrate, but do not respond to human bFGF. In the undifferentiated state, some HCN-1 cells show MHC class I antigen expression. After differentiation, HCN-1 cells and their processes are MHC class I negative. On the other hand, gamma-interferon stimulation enhances MHC class I expression, but does not induce MHC class II immunoreactivity. Our in vitro data indicate that HCN-1 cells express mixed characteristics, including both neuronal and mesenchymal markers, and are consistent with the suggestion that the HCN-1 cell line resembles an immature neuroepithelial cell precursor with a complex origin.

chromaffin cells were prepared from the adult rat adrenal glands and were stained immunocytochemically and with glyoxylic acid-induced fluorescence histochemistry. Mesencephalic neuronal cultures were taken from rat embryos (E13) and were plated on different substrates. After this procedure, the tyrosine hydroxylase positive neurites were estimated by computer assisted image analysis.

Major Past Findings:

(1) We studied genetically normal cerebellar grafts transplanted into a mutant host environment defective in both cell-cell adhesion molecules and myelination (Quaking mice). The grafts survived and showed generally normal cytoarchitecture with characteristic expression and distribution of neurofilaments. Normal phosphorylation of neurofilaments occurred in grafts. Myelination was normal as well. These grafts formed internal circuits that partially substituted for the absence of the normal complement of afferent inputs. The data support the notion that neurogenesis with alternative connections can occur in transplants.

(2) We have compared the development of cerebellar allografts using intraparenchymal and intraventricular transplantation techniques. Although intraparenchymal grafts were smaller than intraventricular cerebellar grafts, they were better integrated with the host brain in terms of the presence of interconnecting neurites. This occured despite a large glial scar and demyelination of the host brain. The appearance and distribution of phosphorylated and non-phosphorylated neurofilament epitopes resembled that seen in normal postnatal cerebellar development. Abnormal phosphorylation was not observed. Moreover, the organization of synapse-associated antigens was similar. Thus, internal organization of both types of grafts was relatively normal.

(3) We have studied the expression of cell adhesion molecules in mouse cerebellar grafts. We found no significant differences between the development of transplanted cerebellar tissue and the normal (in situ) development of immunostaining patterns of cell adhesion molecules (N-CAM, L1, J1/tenascin, MAG). Since the trilaminar cytoarchitecture of transplanted cerebella was maintained, it is likely that the molecular mechanisms of granule cell migration in normal cerebella are operant in transplanted tissue. Moreover, the expression of the L_2/HNK-1 epitope suggests that this carbohydrate epitope is differentially expressed in the graft situation when compared to in situ grafts. All data indicate that the developmental appearance of cell adhesion molecules occurs independently of the normal complement of afferent inputs, and is consistent with the possibility that the migration of cells from the external to the internal granular layer involves multiple cellular interactions and is present in transplanted tissue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02450-04 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Screening Human Brain DNA For HTLV-I Proviral Sequences Using PCR Amplification

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

PI:	*D. Glovinsky*	*Sr. Staff Fellow*	*NPB, NIMH*
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LAB/BRANCH
Neuropsychiatry Branch

SECTION
Clinical Neuropsychiatry

INSTITUTE AND LOCATION
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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

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

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

Polymerase chain reaction (PCR) amplification has been used to identify retrovirus-like sequences in both multiple sclerosis patients and non-neurologic control patients. Having used PCR to identify a novel endogenous retrovirus-like sequence in human brain DNA, we are now defining its tissue distribution and transcriptional activity. Genomic DNA was extracted from 12 autopsied brains of patients with multiple sclerosis and from 8 without MS. PCR amplification of the DNA was performed, and radiolabeled fragments of cloned sequences were used to probe DNA complementary to RNA from different human organs., as well as from various animals. No correlation between PCR amplification and MS was found. The DNA insert cloned from PCR amplification products differed from any previously described sequence, and most likely represents an endogenous DNA sequence.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: Polymerase chain reaction (PCR) amplification has been used to identify retrovirus-like sequences in both multiple sclerosis patients and non-neuralgic control patients. Having used PCR to identify a novel endogenous retrovirus-like sequence in human brain DNA, we are defining its tissue distribution and transcriptional activity.

Methods Employed: Genomic DNA was extracted from 12 autopsied brains of patients with multiple sclerosis and from 8 without MS. Using oligonucleotide primers obtained from M.E. Truckenmiller, Ph.D., polymerase chain reaction amplification of the brain DNA was performed. The PCR amplified DNA was size-separated by agarose gel electrophoresis. DNA sequence homologous to HTLV-I were hybridized to a radiolabeled HTLV-I DNA probe. Positive samples were subjected to restriction endonuclease (RE) digestion to verify that expected RE sites were evident. Serum specimens from 10 of the 20 patients were checked for the presence of HTLV-I antibody by ELISA and Western blot techniques. PCR amplified sequences from one specimen that demonstrated homology to HTLV-1 were ligated into a plasmid vector that was used to transform competent cells. Sequence analysis of this insert was performed. A radiolabeled fragment of the cloned sequence was used to probe DNA complementary to RNA from human brain, lung, kidney, liver, pancreas, placenta, skeletal muscle, spleen, testes, and uterus, as well as genomic DNA from the monkey, cow, mouse, and chicken rabbit, rat, and yeast.

Major Findings: There was no correlation between PCR amplification and the presence of multiple sclerosis. PCR amplification of HTLV-I-like sequences was present in human brain (MS and control), monkey, cow, mouse, and chicken genomic DNA, and was not present in the DNA from rabbit, rat, and yeast. The DNA insert cloned from these PCR amplification products differed from any previously described sequence in the two major gene bank libraries. It did have some similarities to retroviral sequences. It most likely represents an endogenous DNA sequence. DNA complementary to RNA was PCR amplified from lung, kidney, liver, pancreas and 6 of 8 human brain samples.

Significance to Mental Health Research: Viruses have been proposed to have a causal role in the development of schizophrenia. Confirmation of the presence of genomic DNA sequences with homology to retroviruses in multiple sclerosis would fuel the search for evidence of viral factors in other neuropsychiatric illnesses. The availability of the PCR amplification technique gives molecular biological researchers another powerful tool to better understand the neurobiological basis of human disease. This is one of the first descriptions of such an endogenous sequence with associated gene transcriptional activity.

Projected Course of Project: The results will be prepared for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02453-04 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Brain Dopamine Release and Metabolism After Atypical and Typical Neuroleptics

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

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COOPERATING UNITS *(if any)*

LAB/BRANCH
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SECTION
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INSTITUTE AND LOCATION
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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

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

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

Clozapine, an atypical neuroleptic, does not affect dopamine (DA) neuronal function in the same way the typical neuroleptics do, which may explain why it produces relatively few extrapyramidal side effects. To gain better insight into how these two classes of neuroleptics influence central DA metabolism, we compared DA turnover and release following acute and chronic doses of clozapine and haloperidol. DA turnover was assessed from changes in the concentration of the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). DA release was assessed from the rate of accumulation of 3-methoxy-tyramine (3MT) after administration of a high dose of pargyline, a potent monoamine oxidase inhibitor. Three brain regions were studied: the frontal cortex, the nucleus accumbens and the caudate nucleus.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: The primary objective is to compare the effects of acute and chronic haloperidol and clozapine administration on DA release and metabolism in the frontal cortex and striatum. These two regions receive dopaminergic projections from the ventral tegmentum (A10) and substantia nigra (A9), respectively. Our second objective is to assess the relationship between DA metabolism and DA release, as reflected by the accumulation rate of 3MT, following inhibition of monoamine oxidase by pargyline. We hypothesize that DA metabolism and release can be differentially changed by typical and atypical neuroleptics in different brain regions.

Methods Employed: All studies were performed on male Sprague-Dawley rats. The biochemical analyses were performed by combined gas chromatography/mass spectrometry. New procedures were developed to accurately measure 3MT in the brain. These procedures involve using chemical ionization instead of electron ionization mass spectrometry.

Major Findings: Dose response studies reveal that DA metabolism in the frontal cortex is more sensitive to acute haloperidol as compared to DA metabolism in the striatum. In contrast, both frontal cortex and striatal DA metabolism are equally sensitive to the acute effects of clozapine.

Dynamic measures of dopamine release and turnover showed that the frontal cortex is more sensitive to the effects of neuroleptics on dopamine release than the nucleus accumbens or striatum.

Acute administration of haloperidol increases DA release (as reflected by 3MT accumulation after pargyline) in both the frontal cortex and the striatum. However, the striatum is more responsive than the frontal cortex. In the frontal cortex, acute clozapine, like haloperidol, increases DA release, but, unlike haloperidol, it reduces DA release in the striatum.

Chronic haloperidol continues to stimulate DA release in both the frontal cortex and the striatum, but the degree of stimulation appears to be less than that produced acutely. Thus, there is a partial tolerance to haloperidol-produced DA release.

Chronic clozapine continues to stimulate DA release in the frontal cortex with less indication of tolerance as compared with chronic haloperidol treatment. Chronic clozapine had no effect on DA release in the striatum.

Significance to Mental Health Research: The results of our investigation demonstrate differences between haloperidol and clozapine with regard to their effects on DA release and metabolism in the frontal cortex and striatum. These results may help to explain some of the therapeutic differences between typical and atypical neuroleptics.

Proposed Course of the Project: The method we developed to assess DA release will be employed to evaluate the properties of other neuroleptics, as well as drugs known to stimulate and influence DA metabolism. We also hope to employ this procedure to study the short- and long-term effects of stimulant drugs on frontal cortex DA. The latter may open a new avenue in the understanding of the role of frontal cortex DA neuronal systems in schizophrenia and drug abuse.

We plan to pursue investigations of the significance of stress on neuroleptic effects on brain catecholamines and other biogenic amines.

Publications:

Egan M, Karoum F, Wyatt RJ. Effects of acute and chronic clozapine and haloperidol administration on 3MT accumulation in the rat prefrontal cortex, nucleus accumbens, and striatum. European Journal of Pharmacology 1991; 199: 191-199.

Karoum F, Egan M: Dopamine release and metabolism in the rat frontal cortex, nucleus accumbens, and striatum: A comparison of acute clozapine and haloperidol. Br J Pharmacology 1992; 105: 703-707.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02457-04 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neuropathologic Studies of Post Mortem Brain of Schizophrenic Patients

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

PI:	*J. R. Stevens*	*Medical Officer*	*NPB, NIMH*
Others:			
	M. Poltorak	*Visiting Scientist*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
Clinical Brain Disorders Branch, NIMH (M. Casanova, J. Kleinman); University of Dusseldorf, Germany (P. Falkai, B. Borgerts); Univ. of Virginia Medical School, Charlottesville, Virginia (L. Heimer, G. Alheid)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Aging

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

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

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

The objective of this study is to search for evidence of anomalous innervation or regeneration in specific nuclei of schizophrenic brains. Formalin-fixed, paraffin-embedded, 50 mm matched sections of nucleus accumbens from 8 schizophrenic and 8 age- and sex-matched control brains were used for studies of morphology and possible axonal sprouting. Using a monoclonal antibody against nonphosphorylated neurofilaments, we compared innervation patterns in limbic forebrain of schizophrenic and control brains at matched levels. A slight decrease in axons and a change in axon pattern was seen in the grouped schizophrenic specimens.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: To explore the use of nonphosphorylated neurofilament antibody to trace pathways in limbic forebrain of whole brain sections of patients with schizophrenia and matched controls.

Methods Employed: Formalin-fixed, paraffin-embedded, 50 μm matched sections of nucleus accumbens from 8 schizophrenic and 8 age- and sex-matched control brains were used for studies of morphology and possible axonal sprouting. Using a monoclonal antibody against nonphosphorylated neurofilaments, we compared innervation patterns in limbic forebrain of schizophrenic and control brains at matched levels.

Major Findings: A slight decrease in axons and a change in axon pattern was seen in the grouped schizophrenic specimens.

Proposed Course: In collaboration with Drs. M. Casanova, W. Freed, M. Poltorak, R. Rawlings (NIAA), and Mr. WS Hsieh (medical student), we have examined studying pathways with this dye in specific regions of the limbic forebrain, including the hippocampus, entorhinal cortex, N. accumbens, and bed nucleus of stria terminalis. If the technique proves useful in fresh and formalin-fixed material from normal brains, we will extend the study to anatomically-matched schizophrenic material.

Significance to Mental Health Research: These attempts to find histologic changes should, if successful, contribute to understanding pathogenesis. The hypothesis that absence of gliosis is evidence for a prenatal etiology of schizophrenia makes the question of presence or absence of gliosis especially important.

Publications:

Stevens JR. Kindling of inhibition and psychosis. In: Morrell F, ed. Kindling: The Legacy of Graham Goddard. Boston: Birkhouser, 1991.

Stevens JS, Casanova MF, Hsieh WS, Poltorak M, Rawlings R, Freed WJ. Axon counts in the nucleus accumbens of schizophrenic patients, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02483-04 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Compensatory Neuroplasticity in Stress and Aging

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

PI:	*G. M. Gilad*	*Visiting Scientist*	*NPB, NIMH*
Others:			
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
Department of Pharmacology, Howard University, Washington, D.C. (Y. Tizabi)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Molecular Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

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

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

During aging, many neurons in the septohippocampal cholinergic system degenerate and may cause memory loss. It is possible that hyper-reactivity to stressful stimuli can also accelerate neuron degeneration in this system. Our aim is to learn: (a) whether or not the characteristic stress-induced adaptive changes in cholinergic neurons are altered in aged rats; (b) how age-related changes in neurotransmitter systems converging in the septum influence the septohippocampal cholinergic system; (c) how age-related changes in the regulation of the glucocorticoid hormonal system affect the septohippocampal cholinergic system; and (d) how stress-induced changes in specific stress-responsive proteins, including polyamine metabolism, are involved in neuronal function. The results so far indicate that age-related degeneration of cholinergic neurons is accompanied by compensatory changes in neighboring neurons to prevent the loss of cholinergic neurotransmission, and that high glucocorticoid levels can accelerate the degeneration rate of cholinergic neurons. Our recent studies also indicate that glutamatergic neurons increase their activity in response to stress in a region selective manner. This activation is probably dependent on the activation of ascending neuronal inputs, such as dopamine neurons, that terminate in the septum.

We recently examined the effects of various regimens of lithium chloride treatment on dexamethasone-induced increases in brain polyamine metabolizing enzymes. In contrast to peripheral tissues, where acute lithium treatment suppresses the increase in polyamine biosynthesis, in the brain, only chronic treatment was effective in preventing this increase.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives:

1. To elucidate the effects of aging on the adaptive response of neurons to stressful stimuli as expressed by changes in their neurotransmission function (e.g. regulation of neurotransmitter synthesis, and release and uptake mechanisms).

2. When neuron degeneration occurs, to elucidate the adaptive response of remaining (intact) neurons as expressed not only by functional changes, but also by structural changes (e.g. collateral sprouting).

3. To elucidate the molecular changes important for the ability of neurons to survive (e.g. alterations in stress-associated proteins, and in polyamine metabolism).

Methods Employed:

The rats' behavior in a novel environment is observed in an open field arena. Mild restraint stress is administered for specific intervals in a confined holding chamber.

Cholinergic neurotransmission is characterized by changes in the activity of choline acetyltransferase, choline uptake and acetylcholine release. Changes in the uptake of the neurotransmitters, glutamate, dopamine, norepinephrine, serotonin and GABA, serve as markers for changes in their respective neuronal systems.

Polyamine metabolizing enzyme mRNAs will be characterized and localized by hybridization techniques.

Immunocytochemical localization of choline acetyltransferase and histochemical localization of acetylcholinesterase serve as markers for cholinergic neurons and their terminals in brain sections. Immunocytochemical localization of putrescine with newly developed antibodies is also being used.

The contents of plasma ACTH and corticosterone, and the content of corticotrophic releasing hormone (CRH) in the brain and its binding in the pituitary serve as markers of changes in the regulation of adrenal glucocorticoids.

The contents of plasma epinephrine, norepinephrine and dopamine serve as markers for the peripheral sympatho-adrenomedullary system. Two-dimensional gel electrophoresis of proteins serves to analyze changes in the protein synthetic and degradative machinery of cells.

Several manipulations are used to alter the stress-induced responses, including adrenalectomy or glucocorticoid injections to alter glucocorticoid status, and treatment with lithium.

Major Findings: Results from studies using two inbred rat strains that differ in their reactivity to stressors and in their life spans indicate several changes during aging:

1. There is an ongoing, age-dependent degeneration of septohippocampal cholinergic neurons, which is associated with two principal compensatory changes in remaining cholinergic neurons: (a) hypertrophy of their perikarya and (b) relative increases in the activity of presynaptic markers in their terminals with unchanged regional distribution, suggesting possible collateral sprouting.

2. Age-dependent loss of septal cholinergic neurons precedes loss of hippocampal pyramidal neurons.

3. Loss of pyramidal neurons in the hippocampus is associated with a compensatory increase in muscarinic binding by remaining target hippocampal neurons. The results imply that higher basal and stress-induced activity of septohippocampal cholinergic neurons may be correlated with an accelerated and more pronounced age-dependent degeneration of this cholinergic system. And, in turn, accelerated age-dependent degeneration of susceptible septal cholinergic neurons may lead to a secondary loss of their cholinoceptive target pyramidal neurons in the hippocampus.

4. Hippocamposeptal glutamatergic neurons increase their activity in response to stressful stimuli. This response is probably independent of the glucocorticoid status and is maintained in aged rats.

5. Stress glucocorticoids and other neurotraumas. Increased polyamine synthesis is a characteristic response too. This polyamine response can be prevented by chronic lithium treatment.

Changes in specific proteins after acute stress. Early stress-induced changes in proteins of the rat hippocampus and frontal cortex were analyzed by two dimensional gel electrophoresis immediately after 2 hrs of immobilization stress. In the hippocampus, a 59,000 Dalton (59K) protein was increased in the soluble fraction, but decreased in the synaptosomal fraction; two 63K proteins differing in pI values were decreased in the soluble fraction; and in the nuclear fraction, a 79K protein was increased prominently, while a 68K protein was decreased. In soluble fractions of the frontal cortex, no alterations were apparent in the 59K protein, while similar changes, as in the hippocampus, were noted in the amounts of 79K and 68K proteins. In contrast, the 63K proteins were increased in the frontal cortex after stress. The results demonstrate that rapid and region-selective changes in the

amounts of specific proteins can be induced in the brain by acute application of stressful stimuli; these changes appear to be related to the intensity of the stressful stimulus.

Lithium and Stress-Induced Changes in Polyamine Metabolism: In a recent series of studies, we examined the effects of various regimens of lithium chloride treatment on dexamethasone-induced increases in brain polyamine metabolizing enzymes. In contrast to peripheral tissues where acute lithium treatment suppresses the increase in polyamine biosynthesis, in the brain, only chronic treatment was effective in preventing this increase. These findings indicate a novel brain target for lithium's action and, in turn, provide new avenues for exploring polyamine function in manic-depressive illness.

Significance to Mental Health Research: The neurotransmitter systems under study (cholinergic, glutamatergic, GABAergic, dopaminergic and neuroendocrine systems) are implicated in the pathology of mental disorders. The degree of reactivity of the systems to stressful stimuli could be involved in precipitating symptoms of neuropsychiatric disorders. Comparative studies of the factors regulating the adaptive response of these systems to stressful stimuli, and the effects of age thereof, are urgently needed to advance our knowledge of the biochemical mechanisms involved. These studies may result in improved drug treatments for mental disorders. The possible implication of brain polyamines in mechanisms of mental disorders is novel, and should be explored further.

Proposed Course of Project: The stress response of the septohippocampal cholinergic system and other septal converging systems will be examined in aged rats. The age-related stress response of the neuroendocrine system (corticotropin releasing hormone adrenocorticotropic hormone and glucocorticoids) will be characterized. Further studies of the stress-associated proteins will be pursued, including the polyamine-response.

Publications:

Gilad GM, Gilad V: Polyamines can protect against ischemia-induced nerve cell death in gerbil forebrain. Experimental Neurology 1991; 111: 349-355.

Gilad GM, Gilad VH, Wyatt RJ, Casero RA, Jr. Chronic lithium treatment can prevent the dexamethasone-induced increase of brain polyamine metabolizing enzymes. Life Sci 1992; 50:18; 149-154.

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

PROJECT NUMBER
Z01 MH 02484-04 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of the 3H-GBR Receptor Sites in the Frontal Cortex

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

PI:	*A. Hitri*	*Sr. Staff Fellow*	*NPB, NIMH*
Others:			
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
Clinical Brain Disorders Branch, NIMH (M. Casanova, J.E. Kleinman); Department of Psychiatry, Medical College of Georgia (H.Q. Nygen)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Aging

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

X(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.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02541-03 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Computed Determination of Gait Abnormalities in Rats with CNS Lesions

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

PI:	*L. de Medinaceli*	*Consultant*	*NPB, NIMH*
Others:			
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews

_(b) Human tissues

x(c) Neither

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

We are developing a method for evaluating the motor deficits and the proprioceptive (sensory) condition of rats with CNS lesions. This method is based on an automatic evaluation of gait performance as rats progress across a grid. A modified version of the apparatus is currently being tested to determine the reliability of the system.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: We intend to explore gait abnormalities in laboratory animals that present with CNS deficits and, ultimately, to compare this data with human data.

Methods Employed: Past methods were based on clinical scoring of motor and sensory conditions, and were therefore subject to imprecision and observer bias. To correct for this, we have developed a method for automatically assessing the walking performance of rats progressing across a grid.

Our method employs the activation of a photoelectric system when placement of the foot is incorrect. From pilot observations, it is expected that this apparatus will allow determination of a reliable index of gait impairment.

The apparatus will be tested on normal and pathological animals. Animals will be tested before and after lesioning. The first lesion to be examined will be a thoracic spinal cord injury, since this type of damage is reproducible and yields functional deficits of a consistent pattern. Then, based on the model of the "deficit state", the apparatus will be tested with rats given 10 mg/kg cocaine twice a day for 1 week.

Significance to Biomedical Research and to the Program of the Institute: Gait abnormalities exist in some neuropsychiatric patients. It is not clear whether these abnormalities are strictly behavioral or whether they have an anatomical-physiological bias. The possibility of assessing similar abnormalities using an animal model may provide some information on the subject.

Major Findings: During the last year, we compiled data with normal animals and tried to determine the normal pattern of behavior. A large number of tests were necessary because of the normal variability of responses. These tests demonstrated that it was necessary to modify equipment to obtain better data. The modified version of the apparatus is currently being used to accumulate new data.

Proposed Course: During the next few months we will determine the reliability of the new system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02543-03 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Relevance to Schizophrenia of Dopamine Transporter Binding in the Frontal Cortex

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

PI:	*A. Hitri*	*Sr. Staff Fellow*	*NPB, NIMH*
Others:			
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
Clinical Brain Disorders Branch, NIMH (M.F. Casanova, J.E. Kleinman, D.R. Weinberger)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Aging

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews

x(b) Human tissues

_(c) Neither

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

This project has been terminated.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02544-03 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Patient Response to Resumption of Neuroleptics Following a Drug Free Interval

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

PI:	*D. Glovinsky*	*Sr. Staff Fellow*	*NPB, NIMH*
Others:			
	R. J. Wyatt	*Chief*	*NPB, NIMH*
	D. G. Kirch	*Guest Researcher*	*NPB, NIMH*

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

X(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 shown that schizophrenics respond to the reinstatement of neuroleptics in a characteristic pattern, but there is disagreement about the mechanism underlying symptom change. To clarify this issue, double-blind, serial measurements of the behavior and symptom change of schizophrenic subjects who resumed neuroleptic medication after a drug-free period were analyzed. Fifty percent of the response occurred by the seventh day of post-drug reinstatement, including a significant improvement in symptom/behavior inventory subscales measuring delusions, hallucinations, and other positive symptoms associated with psychosis.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: A number of studies have shown that schizophrenics respond to the reinstatement of neuroleptics in a characteristic pattern. There is some disagreement about the mechanism of neuroleptic effects upon symptom change. Double-blind serial measurements of the behavior and symptom change in schizophrenic subjects who resumed neuroleptic medication after a drug-free period were analyzed to clarify this issue.

Methods Employed: The data set consisted of serial measurements of the response by a set of schizophrenics who, while inpatients at the Neuropsychiatric Research Hospital, recommenced neuroleptic pharmacotherapy after a 6-week drug-free period. The measurements were undertaken in a double-blind fashion. A standardized research behavior/symptom inventory was used. The Statistical Applications Service (SAS) program was used to analyze the data set. Patient response to remedication was analyzed by the change in the total symptom inventory score as well as in the subscales.

Major Findings: The data was analyzed in graph form. Fifty percent of the response occurred by the seventh day of post-drug reinstatement. This included a significant improvement in those symptom/behavior inventory subscales that measured delusions, hallucinations, and other positive symptoms associated with psychosis.

Significance to Mental Health Research: Although neuroleptic medication has been used to treat schizophrenic patients for over 20 years, the mechanism by which alteration of symptoms occurs is not understood. By closely examining the pattern of response by schizophrenics to neuroleptics, a more accurate description of this process can be rendered. Explanatory hypotheses can be tested against using data, thereby allowing a more accurate conceptualization of the mechanism of action of these drugs, and more effective and efficient treatment of schizophrenics with neuroleptic medication. The early response of psychotic patients described here may challenge some current models of neuroleptic pharmacology.

Proposed Course of Project: The project results will be prepared for publication.

Publication:

Glovinsky D, Kirch D, Wyatt RJ. Early anti-psychotic response to resumption of neuroleptics in drug-free chronic schizophrenic patients, Biological Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02545-03 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Symptom Checklist for Diagnosis of Schizoaffective Disorder

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

PI:	*R. S. El-Mallakh*	*Medical Staff Fellow*	*NPB, NIMH*
Others:			
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
Computer Programmer, NIMH Neuroscience Center at Saint Elizabeths (I. Waldman)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

x(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.)*

This project has been terminated.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02546-03 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Clinical Trial of Clonidine in Patients with Schizoaffective Disorder

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

PI:	*R. S. El-Mallakh*	*Medical Staff Fellow*	*NPB, NIMH*
Others:			
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

X(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.)*

This project has been terminated.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02547-03 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physical Stress and Brain Function

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

PI:	*R. S. El-Mallakh*	*Medical Staff Fellow*	*NPB, NIMH*
Others:			
	G. M. Gilad	*Visiting Scientist*	*NPB, NIMH*
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

X(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.)*

Since motor hyperexcitation is a frequent manifestation of psychotic states, the question has been raised as to whether physical hyperactivity may increase brain dopamine, thereby contributing to the brain dysfunction frequently seen in schizophrenia. In an attempt to answer this question, a rat model is being developed. The model would involve forced wheel running of Brown-Norway rats to the point of exhaustion. Recovery would be monitored by open field behaviors. Biochemical studies involving dopamine, its metabolites, and other neurochemicals would help delineate the role of physical hyperactivity and concomitant stress on brain function. The wheel running apparatus has been constructed and the protocol has been approved by the ACUC.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: To study the neurochemical effects of exhaustive physical stress on Brown-Norway rats.

Methods Employed: Will develop an animal model based on forced wheel running and open field behavior. Specifically, previous work has shown that a fraction of female rats that are forced to run in a wheel until fatigued may have a permanent change in their behavior. These changes appear to be related to changes in catecholamine metabolism. Previous workers have not continued investigation in this area. We would like to investigate the brain effects of extensive physical activity on catecholamine systems.

Major Findings: Development of the model is still in progress.

Significance to Medical Research: This protocol may serve as an animal model of the agitation and hyperactivity that may be associated with psychosis. As such, it would be a valuable tool in understanding the effects of this state on neuronal functions.

Proposed Course: Once the model is developed, neurochemical and pharmacologic studies can be conducted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02548-03 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Carbidopa in the Treatment of Psychotic States

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

PI:	*R. S. El-Mallakh*	*Medical Staff Fellow*	*NPB, NIMH*
Others:			
	F. Karoum	*Chemist*	*NPB, NIMH*
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0

CHECK APPROPRIATE BOX(ES)

X(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 work in our Branch and elsewhere has shown that in some individuals psychosis is accompanied by significant elevations in the amount of phenylethylamine (PEA) excreted in the urine. PEA is a metabolite of phenylalanine and is normally produced in both peripheral and central nervous systems. It is rapidly degraded by monoamine oxidase. PEA is known to have many behavioral similarities to amphetamines when injected into rats. In a few individuals with psychosis and elevated urinary PEA, the administration of carbidopa (an L-aromatic amino acid decarboxylase inhibitor) reduces excreted PEA and ameliorates the psychosis. We will identify patients with elevated PEA by screening their urine, and will then ask them to participate in a protocol that will compare carbidopa and neuroleptic effects in a double-blind fashion. The study has been approved by the IRS. Over 40 patients have been screened, but only two people had elevated urinary PEA. Unfortunately, both refused to participate in the study. The protocol is being modified to allow for wider selection of patients. Screening is being expanded.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: To examine the role of phenylethylamine, an endogenous, trace amine, in the genesis of psychosis, and to examine the efficacy of a potentially useful, novel therapeutic agent.

Methods Employed: Screening of psychotic patients' urine for elevated levels of phenylethylamine (PEA). This will be accomplished with three consecutive morning urine samples and PEA quantified with mass fragmentography. Patients with elevated PEA levels will be identified and given the option of participating in a double-blind, placebo-controlled trial of Carbidopa. Carbidopa is a peripheral L-aromatic amino acid decarboxylase inhibitor and will, therefore, decrease peripheral decarboxylation of phenylalanine to PEA. This will presumably decrease PEA entry into the CNS and indirectly decrease CNS PEA.

Major Findings: Patient screening continues.

Significance to Medical Research: This study may identify a distinct biological category of psychosis and may also lead to a specific treatment released patients.

Proposed Course: Patient screening and recruitment will continue. Study will proceed as per protocol.

Publications:

El-Mallakh RS, Kirch DG, Shelton R, Kuang-Jaw F, Pezeshkpour G, Kanhouwa S, Wyatt RJ, Kleinman JE. The nucleus basalis of Meynert, senile plaques, and intellectual impairment in schizophrenia, Journal of Neuropsychiatry and Clinical Neurosciences 1991; 3: 383-386.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02549-03 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Infection of Rabbits with Human Immunodeficiency Virus I

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

PI:	*H. Kulaga*	*Research Biologist*	*NPB, NIMH*
Others:			
	A. J. Adams	*Biologist*	*NPB, NIMH*
	M. A. Coggiano	*Biologist*	*NPB, NIMH*
	M. E. Truckenmiller	*Sr. Staff Fellow*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
Nova Pharmaceuticals, Baltimore, MD (P.M. Sweetnam); LIG, NIAID (T.J. Kindt); CDC, Atlanta, Georgia (T.M. Folks)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Molecular Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  _(a2) Interviews

x(b) Human tissues

_(c) Neither

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

This project focuses on characterization of HIV-I infection of the CNS, and combines the fields of molecular biology, virology and immunology. These studies entail the use of a human in vitro neuronal cell line (HCN-IA), and the rabbit in vivo system. With these systems, alternate viral receptors (nonCD4) and their functions in HIV-I infection, latency and damage within the CNS are further defined.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: It has been shown by several criteria that rabbits can be infected with HIV-1 when animals are administered HIV-1 infected human cells. Current studies aim to define the course of infection in the rabbits, including any pathological effects, as determined by macroscopic and microscopic methods with particular emphasis on the CNS. Functional changes in immunity following viral infection will also be investigated. Furthermore, certain therapeutic agents known to block HIV-I infection of a human neuronal cell (HCN-IA) will be tested for their ability to inhibit infection in the rabbit.

Methods Employed: Rabbits are infected by an intravenous injection of HIV-1 infected human A3.01 cells. Antibodies to viral proteins are detected by commercial HIV-1 ELISA kits that have been adapted to measure rabbit antibodies. Immunoblots are likewise carried out using strips with recombinant HIV-1 proteins and reagents for the detection of rabbit antibodies. The virus is detected by the presence of reverse transcriptase activity and p24 antigens in cultures of cells from infected rabbits. The polymerase chain reaction is used to detect viral nucleic acids in cells and tissues from infected rabbits. Primers are chosen from regions that are highly conserved in the various isolates of HIV-1 for which sequence data are available. Areas in the gag and env genes have been chosen since these regions are at opposite ends of the viral genome. Active viral replication is also measured by in situ hybridization using HIV-1 probes prepared in a vector that produces RNA. Immune function in infected rabbits is measured by ELISA for antibody to tetanus toxoid (TT) and skin reaction for BCG. Tests to measure cytokine production by rabbit lymphoid cells in response to various general and specific stimuli are being developed.

Major Findings: It is possible to reproducibly infect rabbits with HIV-1, using a protocol that involves injection of the animal with A3.01 cells that are equivalent to 10,000 infectious units ,as determined by an indirect plaque-forming assay. Antibody is detectable after 4 weeks, and, after 6 weeks, the virus is usually detected in the spleen and lung by PCR; prior to that time it cannot be detected. Evidence for HIV-1 infection in a number of brain regions was also obtained by molecular analysis, the most commonly infected area being the thalamus. HIV-1 could be detected as early as 4 months post-infection and as late as 1 year post-infection. Nucleic acid sequence analysis shows an 80% homology to LAV (original inoculum). Further investigation will compare splenic isolates with CNS isolates in the rabbit. Preliminary sequence analysis of HIV-1 gag and env fragments indicated a close homology to the virus detected in lymphoid tissue of the same animal. In situ hybridization was used to locate viral transcripts in the spleens, lungs, appendixes, skin, and brains of infected rabbits. Viruses can be detected in cultured PBMC by reverse transcriptase assays and p24 antigen capture. Rabbits immunized with BCG and TT were infected with HIV-1, and immune responses were measured. Skin reactivity to PPD, and antibody titers to TT were markedly depressed as compared to

control animals. The fact that no disease was seen in rabbits infected with HIV-1 alone (rabbits infected with both HTLV-1 and HIV-1 showed some signs of illness) may be because the rabbits were maintained under specific pathogen-free conditions and were therefore not exposed to the pathogens that most commonly cause disease in rabbits.

Significance to Mental Health Research: Important questions regarding the utility and feasibility of the use of the rabbit as a model for HIV-1 infection are addressed in this work. The first question is the amount of inoculated virus required to establish productive infection in the rabbit. In other model systems of HIV-1 (or SIV) infection, the exact amount of infectious material necessary to induce infection is known. With this information, desired effects and, more importantly, well-planned strategies for vaccine testing can be proposed. Since a minimal rabbit infectious unit has been determined (10,100 units), drug or vaccine testing with the species will become more realistic. Sequence analysis of rabbit brain and splenic amplification products versus original inoculum will give valuable information regarding host range and tissue tropism.

The second aim of this proposal concerns characterization of nonCD4-mediated mechanisms of CNS protection. Since HCN-1A (which is permissive to HIV-1) does not express CD4 at the molecular or cellular level, and infection of the rabbit is not solely CD4-dependent, a combination of these two systems will be used to determine the efficacy and action of a variety of neuroprotective agents.

Proposed Course: The basic materials and methods for in vitro and in vivo infections are described. These experiments will extend observations made with a primary human neuronal cell line (HCN-1A) in culture, under a variety of infectious conditions (ie. viral strain, pharmacologic agents), to a living system (the laboratory rabbit).

Publications:

Recker DP, Kulaga H, Dorsett D, Folks T, Kindt TJ. A monocyte-derived factor interferes with detection of reverse transcriptase in HIV-1 infection, AIDS Res. and Human Retroviruses 1991;70:73-81.

Kulaga H, Recker D, Kuta E, Hague BF, Truckenmiller ME, Coggiano M, Shen Y, Lock A, Kindt TJ. The brain is a target for HIV-1 infection in the rabbit, AIDS Res. and Human Retroviruses 7;1991:165.

Coggiano MA, Alexander RC, Kirch DG, Wyatt RJ, Kulaga H. The continued search for evidence of retroviral infection in schizophrenic patients. Schizophrenia Research 1991; 5: 243-247.

Kulaga H, Truckenmiller M, Coggiano M, Shen Y, Adams A, Recker D, Kuta E, Kindt T: HIV-1 infection of the central nervous system in the rabbit. Journal of Experimental Medicine, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02567-02 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Corticosteroids, Stress and Synaptosomal Glutamate Uptake

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

PI:	*R. S. El-Mallakh*	*Medical Staff Fellow*	*NPB, NIMH*
Others:			
	G. M. Gilad	*Visiting Scientist*	*NPB, NIMH*
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

x(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.)*

Moderate stimuli will cause an increase in synaptosomal glutamate and choline uptake in the hippocampus, septum, frontal cortex, and basal ganglia of the rat. These increases occur after serum increases in endogenous corticosteroids. Consequently, the role of corticosteroids in modulating this brain change is of interest.

Experiments with adrenalectomized rats, dexamethasone-challenged rats, and in vitro preparations suggest that glucocorticoids do not play a role in stimuli-related changes in glutamate uptake.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: To delineate the role of corticosteroids in stress-related increase in synaptosomal glutamate and choline uptake.

Methods Employed: Restraint stress in adrenalectomized rats will be used. Glutamate and choline uptake will be tested in crude synaptosomal fractions.

Major Findings: Thus far, no corticosteroid effects on glutamate or choline uptake have been found.

Significance to Medical Research: This work will contribute to the understanding of mechanisms of brain response to stress and the interface of neurotransmitter and neurohormonal interactions.

Proposed Course: If the experiments continue to show a lack of corticosteroid effect, they will be terminated.

Publications:

El-Mallakh RS. The use of clonidine in psychiatry, Resident and Staff Physician 1992; 38(2): 29-34.

El-Mallakh R, Tasman A: Recurrent abortions in a bulimic: Implications regarding pathogenesis. International Journal of Eating Disorders 1991; 10: 215-219.

El-Mallakh R, Kranzler H, Kamanitz J: The association between headaches and alchohol and drug abuse in substance abuse patients. Headache Quarterly, Current Treatment and Research 1991; 1: 319-322.

El-Mallakh R, Kranzler H, Kamanitz J: Headaches and psychoactive substance use. Headache 1991; 31: 584-587.

El-Mallakh R, Wilson J, Silverman R: Beekeeping for entomological teaching. The Science Teacher 1991; 58: 26-31.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02568-02 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Dopamine Transport Receptor in the Frontal Cortex of Cocaine Addicts

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

PI:	*A. Hitri*	*Sr. Staff Fellow*	*NPB, NIMH*
Others:			
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
Clinical Brain Disorders Branch, NIMH (M.F. Casanova, J.E. Kleinman)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Aging

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

x(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.)*

This project has been terminated.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02595-01 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Trial of Combined Neuroleptic and Selegiline for Treatment of Schizophrenia

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

PI:	*A. Sambunaris*	*Medical Officer*	*NPB, NIMH*
Others:			
	A. Elkashef	*Commissioned Corp*	*NPB, NIMH*
	M. Egan	*Sr. Staff Fellow*	*NPB, NIMH*
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

x(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.)*

To study the effects of augmenting central dopamine activity on deficit state symptoms, we have proposed a sixteen week, double-blind, placebo-controlled trial of selegiline given to patients on a stable dose of neuroleptics. It is hoped that selegiline, a selective MAO-B inhibitor that increases the amount of dopamine, will alleviate negative symptoms without concurrently exacerbating the positive symptoms and side-effects associated with typical non-selective MAO-inhibitors.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: To study the effects of augmenting central dopamine activity on "negative" or "deficit" state symptoms, we have proposed a sixteen week, double-blind, placebo-controlled, trial of selegiline (L-deprenyl) administered to patients on a stable dose of an antipsychotic medication.

Methods: Each subject will serve as his or her own control in a double-blind, placebo-controlled, randomized crossover trial. Patients having an SCID diagnosis of schizophrenia who have been on a stable dose of neuroleptics for at least six weeks will be included in the study. While on the neuroleptic, each patient will undergo an eight-week period of treatment with selegiline, and an eight-week treatment period with placebo. Patients will be rated twice a day with the PSAS, and will be blindly rated twice each week with the SANS and NSRS. In addition, staff will administer weekly AIMS examinations, and neuropsychological testing at the end of each treatment period.

Major Findings: We will commence the study after receiving final IRB approval of the protocol.

Significance to Biomedical Research: Attempts to use dopamine agonists to alleviate negative symptoms of schizophrenia have resulted in a global worsening of symptoms rather than an improvement. Administration of non-selective MAO-inhibitors to schizophrenics receiving neuroleptics has also been contraindicated. We hope that selegiline, a selective MAO-B inhibitor that increases the amount of dopamine available, will ameliorate negative symptoms without concurrently exacerbating the positive symptoms and side-effects associated with typical non-selective MAO-inhibitors.

Proposed Course: Patients will be tested over the next year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02596-01 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effect of Coenzyme-Q10 in Schizophrenia and Tardive Dyskinesia

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

PI:	*A. Elkashef*	*Commissioned Corp*	*NPB, NIMH*
Others:			
	M. Egan	*Sr. Staff Fellow*	*NPB, NIMH*
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
Clinical Brain Disorders Branch, NIMH (T. Hyde and J. Gold)

LAB/BRANCH
Neuropsycchiatry Branch

SECTION
Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

X(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.)*

Recent research suggests that free radical scavengers could be effective in the treatment of Tardive Dyskinesia, and that hypometabolism is evident in the frontal areas of schizophrenics. Coenzyme-Q10, a metabolic enhancer and potent free-radical scavenger, could effectively enhance metabolism in the brains of schizophrenics, thus improving their cognitive function and deficit symptoms. It could also prove effective in the treatment of Tardive Dyskinesia. Subjects will undergo a double-blind, placebo-controlled, crossover trial of Co-Q10, as well as assessments with the BPAS, SANS, AIMS, and neuropsychological tests. No results have been obtained thus far, as we are still in the preliminary stages of the study.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: We plan to study the effect of Coenzyme-Q10, a metabolic enhancer and potent free-radical scavenger, in the treatment of schizophrenics with and without Tardive dyskinesia.

Methods Employed: A double-blind, placebo-controlled, crossover trial of Co-Q10 will be employed. Assesment will include the BPAS, SANS, AIMS, and neuropsychological testing.

Major Findings: No results have been obtained thus far, since we are still in the preliminary stages of the study.

Significance to Biomedical Research: Co-Q10 could effectively enhance metabolism in the brains of schizophrenics, thus improving their cognitive function and deficit symptoms.

Proposed Course: The study will be conducted over a period of 2 to 3 years.

Publications: none

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02597-01 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
PET Studies of the Dopamine Neurotransmitter Dependent Tracer 6-^{18}F-DOPA

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

PI:	*A.M. Elkashef*	*Commissioned Corp*	*NPB, NIMH*
Others:			
	R.J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
Lab. Cerebral Metabolism, NIMH (D. Doudet, R. Cohen); NIDCD (A. Braun); Warren G. Magnuson Clin Ctr., Nuclear Med. Dept. (P. Herscovitch)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizaheths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

X(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 schizophrenia and Tourette's disorder will be compared to normal controls with respect to the uptake of the dopamine neurotransmitter-dependent tracer 6-^{18}F-DOPA by positron emission tomography (PET). PET allows quantification and localization of tissue radionuclide content with high spatial resolution. 6-^{18}F-DOPA, an analogue of L-DOPA allows in vivo visualization of dopamine and its metabolites in the areas of the brain believed to be involved in schizophrenia and Tourette's disorder.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: Patients with schizophrenia and Tourette's disorder will be compared to normal controls with respect to the dopamine neurotransmitter tracer 6-^{18}F-DOPA by PET. To determine the effect of medication on DOPA uptake, patients will be studied on and off neuroleptics.

Methods Employed: Intravenous and arterial lines will be inserted
Pat Lak curves and compartment modeling will be employed for data analysis.

Major Findings: We are currently running normal volunteers and, therefore, have no findings to report.

Significance to Biomedical Research: PET allows quantification and localization of tissue radionuclide content with high spatial resolution. 6-^{18}F-DOPA, an analogue of L-DOPA, allows *in vivo* visualization of dopamine and its metabolites. This will allow us to better understand the effect of neuroleptics on dopamine uptake in patients with schizophrenia and Tourette's syndrome.

Proposed Course: So far, 10 normal controls have participated in the study. In the months to come we will be testing schizophrenics on and off neuroleptics as well as patients with Tourette's syndrome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02598-01 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Effects of Cocaine on Dopamine Uptake in the Rat Brain

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

PI:	*J. M Masserano*	*Special Expert*	*NPB, NIMH*
Others:			
	R. J. Wyatt	*Chief*	*NPB, NIMH*
	D. Venable	*Biologist*	*NPB, NIMH*

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Molecular Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews

x(b) Human tissues

_(c) Neither

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

The drug cocaine enhances the action of dopamine in the brain. Humans who take cocaine in high doses for a prolonged period of time may suffer symptoms of prolonged depression and apathy similar to the negative symptoms seen in schizophrenics. Similarly, in rats, the chronic administration of high doses of cocaine can produce a decrease in locomotor activity and a lack of the usual sensitization state seen with lower dose stimulant drug challenge. In the present study, we evaluated the effects of cocaine administration on the uptake of dopamine in three brain areas of the rat. Cocaine (10 mg/kg) was administered intraperitoneally twice a day for 7 days. The animals were sacrificed two weeks after the last injection, and the uptake of ^{3}H-dopamine into synaptosomes from the prefrontal cortex, striatum and nucleus accumbens was examined. Control animals consisted of rats injected on the same schedule with saline. Animals receiving cocaine exhibited a significant decrease in locomotor activity as compared to the saline-treated rats. In addition, the locomotor responses of these animals to a challenge dose of cocaine (2.5 and 5.0 mg/kg) or amphetamine (1 mg/kg) were the same. The uptake of ^{3}H-dopamine into synaptosomes of the prefrontal cortex of cocaine-treated rats was significantly decreased (30%) as compared to the saline-treated rats. There were no significant differences between these groups of rats in dopamine uptake in the striatum or nucleus accumbens. Kinetic analysis of the data indicated that the decrease in dopamine uptake in the prefrontal cortex of the cocaine-treated rats was due to a decrease in the Vmax of the uptake pump for dopamine. GBR12909, a selective inhibitor of dopamine uptake, and cocaine inhibited ^{3}H-dopamine uptake into the prefrontal cortex, striatum and nucleus accumbens in a similar manner. Treatment of rats with methamphetamine, using the same paradigm, did not produce any changes in dopamine uptake in the three brain areas, indicating that cocaine was selective in producing the decrease in dopamine uptake in the prefrontal cortex.

PHS 6040 (Rev. 5/92)

Project Description

Objectives: The purpose of this study was to evaluate the behavioral and biochemical effects of repeated administration of cocaine to rats. We were interested in evaluating whether our regiment of cocaine treatments would produce a change in baseline locomotor activity, and whether we would find any behavioral sensitization in our animals following a challenge dose of cocaine or amphetamine. Biochemically, we were interested in examining the effects of cocaine treatment on dopamine uptake in the prefrontal cortex, striatum and nucleus accumbens. Earlier data from the Neuropsychiatry Branch showed that chronic cocaine treatments produced a decrease in dopamine and DOPAC levels, as well as GBR 12909 binding, in the prefrontal cortex, but not in the striatum. This study expands on these previous studies by examining the physiological uptake of dopamine into nerve terminal regions after cocaine administration.

Methods: Rats were injected intraperitoneally with cocaine twice daily for seven days (10 mg/kg). Control animals received similar treatment with saline. Two weeks after the last treatment, the animals were sacrificed under halothane anesthesia and three brain areas were removed (striatum, nucleus accumbens, prefrontal cortex). Synaptosomes were prepared from these brain areas and ^{3}H-dopamine uptake into the synaptosomes was measured by filtration assay.

Major Findings: Animals treated chronically with cocaine showed a significant decrease in locomotor activity as compared to the saline-treated controls. Saline- and cocaine-treated animals showed significant increases in locomotor activity when challenged with cocaine (2.5 and 5.0 mg/kg) and amphetamine (1.0 mg/kg); however, there were no significant differences between these two groups of animals in the maximum response to these stimulant drugs. The uptake of ^{3}H-dopamine in the prefrontal cortex was significantly decreased in the cocaine-treated rats as compared to the saline-treated rats. There were no differences between the saline- and cocaine-treated rats in ^{3}H-dopamine uptake in the striatum or nucleus accumbens. The decrease in ^{3}H-dopamine uptake in the prefrontal cortex of the cocaine-treated rats was due to a decrease in the Vmax of the uptake pumps for dopamine with no change in the Km of the uptake pumps for dopamine. Treatment of rats with methamphetamine (10 mg/kg twice a day for 7 days) produced no changes in ^{3}H-dopamine uptake in the prefrontal cortex, striatum or nucleus accumbens as compared to saline-treated rats.

Significance to Biomedical Research: This work aids in the establishment of an animal model for the negative symptoms seen in schizophrenia. Schizophrenics are known to exhibit negative symptoms that are similar to symptoms exhibited by addicts who have withdrawn from cocaine. These symptoms include depression, apathy and lack of motivation. We have found a depression of locomotor activity in

our cocaine-treated rats which may correlate with the depressed mood of cocaine addicts after withdrawal. Biochemically, this depressed locomotor activity may be related to the down regulation of dopamine uptake pumps in the dopamine neurons located in the prefrontal cortex of the rat. This does not occur in subcortical regions of the brain (nucleus accumbens or striatum), and does not occur following similar treatment with the stimulant drug methamphetamine. This indicates that cocaine produces a selective inhibition of the prefrontal cortex that is different from other stimulant drugs.

Proposed Course: This work verifies earlier studies performed in the Neuropsychiatry Branch that found a decrease in the binding of GBR 12909 to dopamine uptake pumps in the prefrontal cortex of cocaine treated rats. Future studies will include the following experiments:

We will attempt to block the decrease in GBR binding in the prefrontal cortex produced by cocaine by using selective receptor agonists and antagonists. The compounds that will be studied include a) SCH 23390, a dopamine D1 receptor antagonist, 2) MK801, a NMDA receptor antagonist, 3) haloperidol, a dopamine D2 receptor antagonist and antipsychotic drug, and 4) clozapine, a dopamine receptor antagonist and atypical antipsychotic drug.

MRNA levels for the dopamine uptake pump will be examine in the cell body regions of the brain that synthesize dopamine uptake pumps, in the ventral tegmental area, and in the substantia nigra.

The findings on the effects of chronic cocaine administration on the uptake of dopamine in the prefrontal cortex will be extended to include the effects of cocaine on the uptake of dopamine into slices made from the dopaminergic cell body regions, ventral tegmental area and substantia nigra.

Publications:

Masserano, J.M, Venable D. and Wyatt R.J: Effects of the Repeated Administration of Cocaine on ^{3}H-Dopamine Uptake into Synaptosomes from the Prefrontal Cortex, Nucleus Accumbens and Striatum of the Rat, in preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02599-01 NPB

PERIOD COVERED: October 1, 1991 to September 30, 1992

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

The Effects of Cocaine on Tyrosine Hydroxylase Activity in the Rat Brain

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

PI:	J. M. Masserano	Sepcial Expert	NPB, NIMH
Others:			
	R. J. Wyatt	Chief	NPB, NIMH
	I. Baker	Biologist	NPB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH: Neuropsychiatry Branch

SECTION: Molecular Neuropsychiatry

INSTITUTE AND LOCATION: NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews

x(b) Human tissues

_(c) Neither

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

Dopaminergic neurons in the brain are thought to play an important role in the etiology of schizophrenia, which is thought to be due to an imbalance of dopamine in certain brain areas. The drug cocaine enhances the action of dopamine in the brain. Humans who take cocaine in high doses for a prolonged period of time may suffer symptoms of prolonged depression and apathy similar to the negative symptoms seen in schizophrenics. Similarly, in rats the chronic administration of high doses of cocaine can produce a decrease in locomotor activity and a lack of the usual sensitization state seen with lower dose stimulant drug challenge. In the present study, we evaluated the effects of cocaine administration on the activity of tyrosine hydroxylase in five brain regions of rats. Cocaine was administered in a dose of 10 mg/kg intraperitoneally, given twice a day for 7 days. The animals were sacrificed 1 hour and 1, 3, 6, and 12 weeks after the last injection and the activity of tyrosine hydroxylase was measured in the prefrontal cortex, striatum, nucleus accumbens, substantia nigra and ventral tegmental area. Control animals consisted of rats injected on the same schedule with saline. Tyrosine hydroxylase activity was significantly increased in the cocaine-treated animals in the nucleus accumbens (11%) and substantia nigra (14%) one hour after the last cocaine administration. A more substantial increase in tyrosine hydroxylase activity of approximately 50% occurred in the ventral tegmental area at 6 and 12 weeks after the last cocaine administration. There were no other changes in the activity of tyrosine hydroxylase at any other times in the five brain regions. These data indicate that the cell bodies of the ventral tegmental area are showing a delayed response to chronic cocaine treatment as represented by the increase in tyrosine hydroxylase activity.

Project Description:

Objectives: The purpose of this study was to evaluate the activity of tyrosine hydroxylase in dopaminergic brain regions of the rat after chronic cocaine treatment. Since catecholamine levels have been shown to be decreased in the prefrontal cortex following chronic treatment with cocaine, we hypothesized that the activity of the enzyme responsible for the synthesis of catecholamines, tyrosine hydroxylase, would also change after chronic cocaine treatment.

Methods: Rats were injected intraperitoneally with cocaine twice daily for seven days (10 mg/kg). Control animals received similar treatment with saline. One hour and 1, 3, 6 and 12 weeks after the last treatment. the animals were sacrificed under halothane anesthesia, and the striatum, nucleus accumbens, prefrontal cortex, substantia nigra and ventral tegmental area were removed, frozen and assayed for tyrosine hydroxylase activity using the ^{14}C-tyrosine/decarboxylase coupled assay.

Major Findings: Tyrosine hydroxylase activity was significantly increased in the cocaine-treated animals in the nucleus accumbens (11%) and substantia nigra (14%) one hour after the last cocaine administration. Tyrosine hydroxylase activity was increased by approximately 50% in the ventral tegmental area at 6 and 12 weeks after the last cocaine administration.

Significance to Biomedical Research: These data add to our knowledge of the effects of cocaine on the central nervous system. Previous work has shown that chronic cocaine administration produces a decrease in dopamine and dopamine metabolites, a decrease in GBR 12909 binding to the uptake pumps, and a decrease in ^{3}H-dopamine uptake in the prefrontal cortex. We have extended this work to show that concomitant with the decrease in dopaminergic function in the prefrontal cortex there is a corresponding increase in tyrosine hydroxylase activity in the ventral tegmental area. This cell body region projects dopaminergic fibers to the prefrontal cortex, and these cells may be responding to the decrease in catecholaminergic function in the prefrontal cortex by attempting to increase the synthesis of catecholamines. Since we are using the chronic cocaine treatments as an animal model for the negative symptoms of schizophrenia, it will be interesting to evaluate whether postmortem brain tissue from schizophrenics also shows similar changes in tyrosine hydroxylase activity.

Proposed Course: Experiments are in progress to further evaluate the increase in tyrosine hydroxylase activity produced in the ventral tegmental area by chronic cocaine treatment. The experiments are as follows:

1) Kinetics studies will be performed to evaluate whether the increase in tyrosine hydroxylase activity is due to an increase in the Vmax or to a decrease in the Km ofthe enzyme or its cofactor tetrahydrobiopterin.

2) The in vivo hydroxylation of tyrosine will be measured to evaluate whether the activity of tyrosine hydroxylase in the intact rat brain is different from that observed in our in vitro assay after chronic cocaine treatment. The possibility exists that following cocaine treatment the actual synthesis of dopamine in vivo, using the animals own endogenous tyrosine and cofactor, may have changed.

3) Levels of mRNA for tyrosine hydroxylase will be measured in the ventral tegmental area at various times after cocaine administration to evaluate whether the long-term increases in tyrosine hydroxylase activity observed at 6 and 12 weeks after cocaine administration are due to a change in the levels of mRNA that code for tyrosine hydroxylase protein.

4) These studies will be extended to the evaluation of tyrosine hydroxylase in human postmortem tissue obtained from normals and schizophrenics.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02600-01 NPB

PERIOD COVERED
October 1, 1991 to June 31, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interleukin-2 in Cerebrospinal Fluid of Patients with Schizophrenia

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

PI: R.S. El-Mallakh Medical Staff Fellow NPB, NIMH

Others:

R.J. Wyatt Chief NPB, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

X(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-2 (IL-2) is a cytokine and growth factor present in the CNS. Previous preliminary work done elsewhere has found IL-2 to be elevated in medication-free schizophrenic subjects. Since elaboration of central growth factors may have significant implications on the pathophysiology of schizophrenia, it was felt that confirmation of this finding would be important. CSF samples from medicated and medication-free schizophrenic subjects and controls were assayed using ELISA for IL-2. It was found that the levels of IL-2 in the human CSF are at the lower detection limits of this assay, and, consequently, there was great variance in the values obtained. Since the assay we used was identical to that used in previous studies, our data suggest that reported IL-2 elevations may be methodological artifacts.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: To replicate previous findings that interleukin-2 (IL-2) may be elevated in medication-free schizophrenic patients.

Methods Employed: An ELISA assay sensitive to 98 pm/cc was used. This assay is identical to that employed by other investigators.

Major Findings: The levels of IL-2 in human CSF are at the lower detection limits of the assay. At these minute quantities the reliability of the assay diminishes. Variance in split samples was equivalent to the differences between groups. This suggests that previously reported elevations of IL-2 in schizophrenics are methodological artifacts.

Significance to Medical Research: Elevations in growth factors, like IL-2, may be a reflection of an ongoing degenerative process with secondary regrowth or primary aberrant neuroplasticity. The identification of these processes in schizophrenia would greatly increase understanding of the disease.

Proposed Course: Our finding will be presented at a national meeting an published. The use of a more sensitive bioassay is being investigated.

Publications:

El-Mallakh RS: HIV-related psychosis [letter]. J Clin Psychology, in press.

El-Mallakh RS: Mania in AIDS: A review of clinical experience and theoretical consideration. International Journal of Psychiatry Medicine 1991; 21: 383-391.

El-Mallakh RS: Sodium-calcium exchange with lithium action [letter]. Journal of Clinical Pharmacology 1991; 11: 279.

El-Mallakh RS: Mania, paranoia and HIV-1. Psychosomatics 1991; 32: 362.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02601-01 NPB

PERIOD COVERED
October 1, 1991 to June 31, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Dopamine-Responsive Growth Factor in Schizophrenia

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

PI:	*R. S. El-Mallakh*	*Medical Staff Fellow*	*NPB, NIMH*
Other:			
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
Department of Neurology, Rush University Medical School, Chicago, IL (P. Carvey)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

X(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 dopamine-responsive growth factor has recently been identified. Findings that the activity of this growth factor is increased in the CSF of patients with Parkinson's disease and in brain extracts of neuroleptic-treated rats suggest that damage or perturbation of the dopaminergic system may be related to elaboration of specific growth factors. Since a possible abnormality of the dopaminergic system has been implicated in schizophrenia, and since neuroleptic (dopamine blocking) drugs are nearly always used in its medical treatment, we elected to examine the activity of this growth factor in the CSF of medicated and unmedicated schizophrenic subjects and normal controls. Our data shows no difference in dopamine-responsive growth factor activity in the schizophrenic group compared to controls. Since we could not replicate the observed neuroleptic effect in brain extracts of rats, it is concluded that the assay, in its current form, is not sensitive enough to detect subtle elaborations of this growth factor.

PHS 6040 (Rev. 5/92)

Project Description:

Objective: To investigate the possible disturbance of the dopaminergic system in schizophrenia by measuring dopamine-responsive growth factor activity.

Methods Employed: A bioassay utilizing concentrated CSF and primary dopaminergic neuronal culture was used. The dopamine-responsive growth factor selectively increases survival of these neurons in cell culture. The number of surviving neurons after 72 hours is a reflection of the activity of the growth factor.

Major Findings: Current methods employed to measure concentrations of the growth factor in CSF appear inadequate.

Significance to Medical Research: The direct investigation of a dopamine-system-specific growth factor would be a tremendous resource in the investigation of many brain diseases, in particular, schizophrenia.

Proposed Course: Methods to improve the concentration of the factor in CSF are being pursued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02602-01 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cerebrospinal Fluid Oxytocin in Schizophrenic and Control Subjects

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

PI:	*D. Glovinsky*	*Sr. Staff Fellow*	*NPB, NIMH*
Others:			
	D. G. Kirch	*Guest Researcher*	*NPB, NIMH*
	R. J. Wyatt	*Chief*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
Clinical Neuroendocrinology Br., NIMH (K.T. Kalogeras, P.W. Gold)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

x(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.)*

Preclinical studies have suggested a link between the neuropharmacology of oxytocin (OT) and changes in behavior, cognition, and neurochemistry associated with schizophrenia. To test these observations clinically, we measured the cerebrospinal fluid OT concentrations of 40 schizophrenic and 15 control subjects using a competitive radioimmunoassay. The CSF OT concentrations of neuroleptic-treated patients were not statistically different from those of the neuroleptic-withdrawn group. No significant differences were found between mean values of the schizophrenic group and the healthy group.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: Preclinical studies have suggested a link between the neuropharmacology of oxytocin (OT) and the changes in behavior, cognition, and neurochemistry associated with schizophrenia. In order to test these observations clinically, we measured cerebrospinal fluid (CSF) OT concentrations in schizophrenic patients and healthy control volunteer subjects.

Methods Employed: Using a competitive radioimmunoassay, OT concentrations in the CSF were determined for forty patients who met DSM-III or DSM-III-R criteria for schizophrenia, and for 15 healthy control subjects. Statistical comparisons of the CSF OT concentrations were made between 1) the neuroleptic-treated and neuroleptic-withdrawn patients and 2) the schizophrenic patient group and the healthy control group.

Major Findings: The CSF OT concentrations of neuroleptic-treated patients were not statistically different from the neuroleptic-withdrawn patient group. There was no significant difference between the mean values of the schizophrenia patient group and the healthy control group.

Significance to Mental Health Research: Although oxytocin has been show to have important effects on cognition, behavior, and social functioning in animals, its role in the neuropharmacology of schizophrenia is less clear. These data suggest that schizophrenia is not associated with detectable quantitative changes in concentration of CSF OT.

Proposed Course of the Project: The results of this study will be prepared for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02603-01 NPB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Diagnosis and CD5+ β-Cells in Psychiatric Patients

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

PI:	*D. Glovinsky*	*Sr. Staff Fellow*	*NPB, NIMH*
Others:			
	H. Kulaga	*Research Biologist*	*NPB, NIMH*
	G. Jackson	*Stanley Fellow*	*NPB, NIMH*
	L. Muhammad	*Stanley Fellow*	*NPB, NIMH*
	A. Adams	*Biologist*	*NPB, NIMH*
	R. J. Wyatt	*Chief*	*NPB, NIMH*
	D. Kirch	*Guest Researcher*	*NPB, NIMH*

COOPERATING UNITS *(if any)*
District of Columbia Commission on Mental Health Services, St. Elizabeths Hospital (L. deVeau; S. Kanhouwa); Mental Health Clinical Research Center, Univ. California, San Diego (M.H. Rapaport)

LAB/BRANCH
Neuropsychiatry Branch

SECTION
Clinical Neuropsychiatry

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

x(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 CD5+ β-lymphocyte subpopulation, which has been linked to several autoimmune diseases, has been reported to be increased in a subset of schizophrenic patients. To test the specificity of this finding, we measured the CD5+ β-lymphocyte percentage of total lymphocytes in blood samples from 81 community mental health center patients and 10 controls. Results were analyzed according to diagnosis. The CD5+ β-cell percentage in the substance abuser group was significantly elevated in comparison to all other groups. The percentage in the schizophrenic group was higher than the control group, but this increase was not statistically significant.

PHS 6040 (Rev. 5/92)

Project Description:

Objectives: The CD5+ B-lymphocyte subpopulation, which has been linked to several autoimmune diseases, has been reported to be increased in a subset of schizophrenic patients. To test the specificity of this finding, we measured the CD5+ B-lymphocyte percentage of total lymphocytes in blood samples from community mental health center patients. The results were analyzed according to diagnosis.

Methods Employed: Using fluorescence activated cell scanning (FACS), CD5+ B-lymphocyte percentages of the total lymphocyte count were determined in residual blood samples from 81 community mental health center patients and 10 healthy control subjects. The DSM-III-R diagnoses were obtained from the admission record. To facilitate data analysis, the diagnoses were clustered into six diagnostic groups. The CD5+ B-lymphocyte percentages were compared, using analysis of variance to determine if significant differences were present between diagnostic groups. A pairwise comparison of the means of each diagnostic group was performed post hoc in order to provide more detailed information.

Major findings: The CD5+ B-cell percentage in the substance abuser group was significantly elevated in comparison to all other groups. The CD5+ B-cell percentage in the schizophrenic diagnostic group was higher than in the control group, but this increase was not statistically significant.

Significance to Mental Health Research: The CD5+ B-lymphocyte is believed to play an important role in the pathogenesis of several autoimmune diseases. Given the association of this lymphocyte subpopulation to autoimmune phenomena, the presence of increased numbers of CD5+ B-lymphocytes in schizophrenics, as previously reported, would suggest the existence of aberrant immune function in schizophrenia. Our findings suggest that CD5+ B-cell increases may be a nonspecific marker of immunological abnormality in psychiatric patients. CD5+ B-cell abnormalities in psychiatric patients are not limited to schizophrenia. Of note, this is the first report of elevated CD5+ B-lymphocyte percentages in substance abusers.

Projected Course: The data will undergo further analysis. The results will then be prepared for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00941-12 LBG

PERIOD COVERED
October 1, 1991 to September 30, 1992

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	LBG, NIMH
Other:	J. Joy	Staff Fellow	LBG, NIMH
	G. Johnson	Biologist	LBG, NIMH
	W. Wallace	Special Expert	LBG, NIMH

COOPERATING UNITS *(if any)*
University of Virginia, Charlottesville; Monoclonetics, Houston Texas; Abbott Laboratories, Chicago

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Biochemical Genetics

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeth's Hospital, Washington, DC 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects _x(b) Human tissues _(c) Neither
_(a1) Minors
_(a2) Interviews

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

Biochemical Genetics Group: The Laboratory continues to search for quantitative and/or qualitative disease-associated changes in gene expression and protein function. In this effort, we have identified an 18,000 dalton protein in cerebrospinal fluid which is increased two fold or more in individuals with both Alzheimer's disease (AD) and schizophrenia. This protein has been tentatively identified as a variant of the alpha chain of haptoglobin. In addition, the Laboratory has established two models for understanding the altered protein content that is exhibited by AD-afflicted tissues. Using a well characterized animal model for AD, we have demonstrated an induction of amyloid precursor protein (APP) in the cerebral cortex of rats with lesions of the nucleus basalis of Meynert. This treated cortex represents an *in situ* model for elucidating the normal function of APP in brain. We have also developed a cell culture model of the Alzheimer's disease brain using neuronal PC12 cells that have been treated by heat shock to understand the role of heat shock 70 proteins in the abnormal modifications of APP and tau. After this stress, tau is abnormally phosphorylated to form A68, the primary protein constituent of neurofibrillary tangles. We have also been studying protein alterations associated with mutations affecting neurally regulated circadian pacemakers, as well as conducting studies to identify proteins that are essential for normal circadian function. These investigations will allow us to understand the pathological consequences of these proteins in degeneration of neurons in diseases and normal aging.

PHS 6040 (Rev. 5/92)

Collaborators:

P. Lemkin	Senior Staff	FCRF, NCI
B. Wolozin	Senior Investigator	LCS, NIMHH
S. Paul	Director, DIRP	NIMH
D. Hochstrasser	Senior Staff	Dept. of Med., Univ. of Geneva
D. Pickar	Section Chief	CNB,NIMH
M. Miller	Senior Staff	NCI
D. Hunt	Professor	Dept. Chem., Univ. of Va., VA
M. Menaker	Chairman Dept. Bio.	Dept. Chem., Univ. of Va., VA
T. Wehr	Chief	CPB,NIMH
M. Roberts	Senior Staff	Clemson Univ.
G. Block	Assoc. Prof.	Univ. of Va., VA
V. Haroutunian	Associate Professor	Mt. Sinai
L. Refolo	Senior Scientist	Athena Neurosciences
I. Lieberburg	V.P. Research	Athena Neurosciences
H. Ghanbari	V.P. Research	Molecular Geriactrics, Rockville, MD

PROJECT DESCRIPTION;

The goals of this project are to characterize alterations of gene expression and protein content due to alterations of neuronal function under normal physiological conditions (during circadian rhythms) and during pathological conditions (aging and neurodegenerative disorders). The project has initially characterized altered protein content due to (1) a mutant hamster exhibiting an altered circadian rhythm and (2) Alzheimer's disease (AD) or schizophrenia in CSF and AD-afflicted brain tissues.

Two approaches were used to characterize altered proteins. First, human samples were assayed for anonymous polypeptides which exhibited different levels in disease vs. normals using high-resolution protein separation methods of two-dimensional electrophoresis (2-DE), and the high sensitivity silver stains, introduced by this laboratory. This forward genetics approach is used to search for mutational events in genetic diseases and permits the screening of as many as three thousand cellular or tissue proteins in a single gel. In genomic terms, this is equivalent to the screening of single copy coding regions equal to more than 3 megabases. These techniques provide us with the ability to detect changes in a protein's charge, mass and/or concentration which may result from altered gene expression mutational effects, post-translational modifications or variations in protein processing. These projects are designed to search for protein variations in several tissues which may be associated with diseases of the central nervous system (CNS). A number of years ago the Laboratory published a 2-DE protein maps for spinal fluid proteins and other laboratories published maps for human serum and plasma proteins. We are currently linking these maps with the published literature on protein variations in disease states to create a clinical database. Once the initial efforts are completed, we plan to keep this database up to date through collaborative efforts. We are continuing to attempt to push our sensitivity of protein detection below 0.1 ng. We are also exploring new methods and newly developed computer densitometry and automatic spot matching programs for the analysis of the complex patterns obtained with the two-dimensional electrophoretograms.

Our second approach is to elucidate the pathological role of specific proteins that have been found to be altered in disease. Using the 2D-E approach, we have found (1) abnormal phosphorylation of elongation factor 2, a protein synthesis factor and (2) elevated levels of heat shock 70 proteins in AD postmortem tissues. We are also investigating two proteins that are associated with the pathology of AD, amyloid precursor protein (APP) and tau. We are using an animal model of AD (the subcortically lesioned rat brain) to investigate the role of APP in

normal brain function and to understand the inability of rat brain to produce senile plaques. We are using a cellular model of neuronal stress (heat shocked neuronal PC12 cells) to investigate the role of the heat shock proteins and abnormal phosphorylation of tau in neurodegeneration.

These two approaches (identifying anonymous polypeptides and characterizing known proteins) are used in conjunction to identify and characterize abnormal proteins and their role in normal circadian rhythm and the pathology of neurodegeneration.

LABORATORY MATERIALS

All the human tissue samples were obtained from patients who were diagnosed by our clinical collaborators, using nationally recognized diagnostic criteria. All animal samples were obtained from our collaborators at the University of Virginia, or the Mount Sinai School of Medicine.

LABORATORY PROCEDURES:

The Laboratory utilizes 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. We also perform cellular fractionations, immunoprecipitations, and in vitro translations and phosphorylations, and computerized gel matching and densitometry.

MAJOR FINDINGS:

A. **Observation of Protein Variations in Cerebrospinal Fluid in Diseases of the CNS.**

We have used a CRADA with Abbott Laboratories (ID# MH 009015) to identify altered proteins associated with diseases. Analysis of silver stained two-dimensional (2D) gels of cerebrospinal fluid (CSF) from patients with schizophrenia (SCZ) and Alzheimer's disease (AD) revealed a significant increase in the relative amount of a polypeptide of 18,000Mr and isoelectric point of 6.5 when compared to the appropriate controls. This protein was identified by its electrophoretic characteristics and by immune analysis of Western blots as an isoform of α-2 haptoglobin, provisionally identified as α-2FS haptoglobin. The major biological function of haptoglobin is to bind hemoglobin thus preventing undue loss of iron through urinary excretion. Haptoglobin is also an acute phase reactant and its concentration in plasma is raised after infection or injury. In addition, the CSF proteins 127 and 128 (M_r 40,000, pI 5.7 and 5.9, respectively), which were previously identified by this laboratory as being present in schizophrenic patients (Harrington et al., 1985), were recently identified as fibrin fragments, derived form fibrinogen (Wildenauer et al., 1991), which is also an acute-phase protein. Fibrinogen is also involved in the blood-clotting cascade which has recently been implicated in neuronal damage and loss in the brain. It is possible that these acute phase proteins play a role in the pathophysiology of schizophrenia and AD and they may ultimately help to provide antemortem diagnostic markers for these diseases of the CNS. A patent has been filed concerning the possible diagnostic use of the 18,000 Mr protein found in AD and schzophrenia.

We have established a CRADA with Monoclonetics International (Project #Z01-MH-00941-07) to search for protein markers in dorsal root ganglia trauma, schizophrenia and amyotrophic lateral sclerosis. We have filed a patent on a marker for chronic dorsal root ganglia crush.

B. **Alzheimer's Disease and the Heat Shock Response**

We have been investigating protein alterations in the Alzheimer's disease brain and the similarity of these alterations to those associated with the heat shock response. Heat shock refers to the cellular response to a variety of insults which minimizes damage and allows for the restoration of normal cellular activities after the period of stress. Previously, we found that AD postmortem tissues exhibited elevated levels of heat shock protein 72, a stress protein which has been shown to act co-translationally as molecular chaperones for nascent polypeptides present within the cytoplasm. In order to investigate the response of neurons to stress, we used nerve growth factor-differentiated PC12 cells incubated at either 37° C (control cells) or 45° C (heat shocked cells). We wanted to determine whether any of the abnormal proteins found in AD brains such as APP and tau may be inappropriately modified due to an association with the hsp. The heat shocked cells exhibited several features characteristic of the stress response including a 45% reduction in total protein synthesis, the induction of heat shock protein 72, an overall decrease in protein phosphorylation, and an increased phosphorylation of the protein synthesis initiation factor, eIF-2alpha. The AD brain also undergoes many changes characteristic of the heat shock response which we term the AD stress response. However, elongation factor 2, which we have found to be hyperphosphorylated in AD brain, exhibited no such increase in heat shocked PC12 cells. We have also examined the phosphorylation of amyloid precursor protein (APP). Two dimensional gel separation of immunoprecipitates of [^{32}P]- labeled PC12 cells with two different antibodies revealed four phosphorylated isoforms of APP (120-140kD, 5.3 pI). APP was dramatically dephosphorylated in the heat shocked PC12 cells.

Using the heat shocked neuronal PC12 cells, we also found modifications of the microtubule-associated protein, tau that mimic those present in AD tissues. Under these conditions of heat shock, tau becomes transformed into A68, the major protein constituent of neurofibrillary tangles. We initially identified A68 in the tau immunoprecipitates as a larger polypeptide (M_r 68kD) using a battery of tau antibodies (such as ALZ 50, tau 1, and tau 2). Tau and A68 exhibited a precursor-product relationship during heat shock; as the amounts of tau were reduced, the amounts of A68 were elevated. A68 was produced in the heat shocked cells in the presence of cycloheximide, indicating that the transformation of tau to A68 occurs post-translationally, such as an abnormal phosphorylation (which has been proposed by others previously). Both tau and A68 were characterized as phosphoproteins using [^{32} P] loaded cells. The altered phosphorylation of tau to produce A68 was reversible when the heat shocked cells were allowed to recover for 2 to 16 hours at 37° C. The phosphorylation was inhibited when the cells were incubated with staurosporine, a specific inhibitor of protein kinase C, suggesting that protein kinase C may be responsible for the heat shock induced phosphorylation.

The role of hsp in this abnormal phosphorylation was then investigated. Heat shock cell lysates were immunoprecipitated with antibody to hsp 72 under nondenaturing conditions in which complexes between hsp 72 and nascent polypeptides are kept intact. Tau was identified to be the most abundant co-precipitating protein, indicating that hsp 72 forms a complex with tau during heat shock. Using differential immunoprecipitation with anti-hsp 72, we found that the tau polypeptides that formed such complexes did not undergo the altered phosphorylation to A68. On the other hand, tau which did not form a complex with hsp 72 was phosphorylated to form A68. These results suggest that under conditions of stress (in PC12 cells as heat shock, in the AD brain, perhaps as neuronal degeneration), hsp 72 associates with tau to protect it from inappropriate phosphorylation. Without association with hsp 72, tau becomes abnormally phosphorylated and is transformed into A68, an initial step in the formation of neurofibrillary tangles.

Previously, the relationship between tau and A68 could be studied only in vitro with characterization of the proteins associated with NFT from postmortem tissues. With this cell model we can investigate the kinetics of the phosphorylation within an intact cell, facilitating an identification of the protein kinases and the intracellular processing that lead to this transformation. Further, the heat shock cells can be used to investigate the physiological consequences of the presence of A68 in the cell.

C. **Amyloid Precursor Protein (APP) is Phosphorylated by Phorbol Ester-stimulated Protein Kinase C in PC12 cells.**

We have also used the neuronal PC12 cells to find that APP is phosphorylated by protein kinase C. PC12 cells prelabeled with [^{32} P] were incubated with various protein kinase activators and inhibitors. APP was then immunoprecipitated from cellular lysates and identified on SDS-PAGE. While incubation with either 8-Br-cyclic AMP or the calcium ionophore A23187 had no effect, PMA, a phorbol ester, dramatically stimulated incorporation of [^{32} P] into APP. Conversely, incubation with staurosporine, a specific inhibitor of protein kinase C, reduced any phosphorylation of APP. These results indicate that protein kinase C phosphorylated APP in vivo an event which has been proposed to be involved in the turnover of APP.

D. **Loss of subcortical innervation results in induction of cortical Amyloid Precursor Protein (APP) in an animal model of Alzheimer's disease.**

APP is the precursor for the amyloid peptide which is the major protein constituent of senile plaques in Alzheimer's disease afflicted brain tissues. A central question in AD research is the cellular mechanism by which the APP gives rise to the amyloid peptide. Of relevance to this question is the normal physiological function of APP. Because a large fragment of the APP molecule is secreted, it has been presumed that some function of the protein is intercellular. Therefore, we have investigated its function in the in situ rat brain in which intercellular communication is kept intact. In collaboration with Dr. V. Haroutunian at Mount Sinai School of Medicine, we have studied the expression of APP in a well established animal model of AD. Rats were treated with various neurotoxins to disrupt the subcortical innervation of specific neurotransmitter systems. The resulting cerebral cortex exhibits reduced neurotransmitter markers which mimic similar reductions found in human AD brains. For example, NMDA applied to the nucleus basalis of Meynert (nbM) causes a large reduction in cholinergic markers in the cerebral cortex. We have found that cortices deprived of cholinergic innervation by lesion of nbM respond to the loss with a 3 to 4-fold increase in the synthesis of APP. The induction of APP was specific in that there was no overall increase in gene expression, including glial fibrillary acidic protein, a marker for reactive gliosis. This induction is rapid, occurring within an hour of lesion placement and persistent, lasting at least 45 days after lesion. It is detectable as elevated APP message on northern blots and as increased synthesis of APP polypeptide by *in vitro* run-off assays of polysomes. It is not specific to the nucleus basalis of Meynert. Lesions of the dorsal raphe nuclei and ascending nuclear bundle, which reduce noradrenergic and serotonergic markers in the cortex also elicit the induction of APP. However, other disruptions of cortical function, including reductions of energy metabolism, inhibitors of acetylcholinesterase, and glucocorticoid treatments do not induce APP. These results suggest that the APP induction is a specific response to any loss of subcortical presynaptic neurotransmission in the cortex.

In collaboration with Dr. Steven Ahlers at the Bethesda Naval Medical Center, we produced a reversible, temporary subcortical lesion by placing lidocaine, a calcium antagonist into the nbM. For the first 30 minutes post-lesion, acetylcholine levels were reduced similar to those reductions exhibited by the permanent lesions. However, after 30 minutes and loss of lidocaine inhibition, the amount of cortical acetylcholine returned to control levels. Concommitant with this reversible reduction of cholinergic function, we found that 20 minutes post injection of lidocaine, the APP mRNA levels were elevated approximately 3-fold, similar to neurotoxin lesion-induced values. However, two days after the lidocaine treatment, the levels of APP were indistinguishable from nontreated cortices. These results suggest that the induction of APP is due to loss of synaptic function from the subcortical but not due to the physical loss of the synapse. The reversible induction of cortical APP in response to loss of subcortical innervation suggests that APP may serve some role in synaptogenesis and/or plasticity in response to injury.

Despite the long term induction of APP in the subcortically lesioned rat brains, these cortices do not produce the senile plaques present in AD brains. We found that the mature, fully processed forms of the APP polypeptide were not increased in the cortices even though these same cortices contained increased levels of APP mRNA. This observation indicates that the turnover of the induced APP is increased in the lesioned cortex which would explain the lack of senile plaques. Because the rat brain efficiently catabolizes the excess APP, the beta-amyloid peptide, which forms the plaque, is effectively removed from the cortex. We will examine the turnover of the induced APP and manipulate the APP gene in transgenic animals in order to investigate APP processing and plaque biogenesis.

E. **Proteins Involved in the Generation and/or Regulation of Circadian Pacemakers**

Circadian rhythms are a fundamental element of human physiology. Indeed, human physiology is subserved by rhythms and periodic processes that influence every level of organization, from molecular to behavioral. Disruptions in physiological timing are associated with reproductive, sleep, and mood disorders.

Circadian rhythms are ubiquitous among eukaryotic life and have been extensively studied in many organisms. However, the molecular mechanisms that make up the clock have not yet been defined in any system. Since circadian clocks are thought to be built of proteins, we have been using two-dimensional gel electrophoresis coupled with computer analysis based on the Elsie V program to identify clock proteins.

One of the requisites for identifying proteins that act as essential components of circadian pacemakers is the identification of suitable model systems. Two approaches that have been particularly promising in the identification of the biochemical pathways involved in the generation of circadian rhythms are (i) the study of clock mutants (e.g. *Drosophila*, *Neurospora*, and the tau mutant hamster), and (ii) the study of relatively simple and discrete systems that oscillate *in vitro* (e.g. the Molluscan eye, chick pineal, and the unicellular algae, Gonyaulax). A third approach, although one that has been less exploited due to limitations inherent in available model systems is to study circadian pacemakers as they are undergoing differentiation. In our investigations of the molecular mechanisms that generate circadian rhythms, we have been studying model systems that incorporate each of these three approaches.

The first model involves candidate clock proteins identified in the Golden hamster. We have found two proteins in hamsters with a mutation that causes a large change in the period of the circadian rhythm that differ from proteins in wild type animals (in collaboration with Dr. M. Menaker, University of Virginia). One protein (about 33 kd, pI 6.5) is missing in homozygous mutants and present in heterozygote and wild type animals; the other (about 32 kd, pI 4.8) is present in all genotypes, but appears as a chain of spots in wild type animals and as two discrete spots in both heterozygous and homozygous mutants, suggesting the tau mutation alters the post-translational processing of this protein.

The second model is characterizing clock proteins in Bulla Eye The secondary photoreceptors (basal retinal neurons = BRNs) of the mollusk, *Bulla*, contain a circadian clock. They offer a unique advantage in that they can be dissected apart from other retinal cells and thus, provide a relatively homogenous "clock preparation." The mammalian circadian clock, the SCN, is a very heterogenous structure and currently exceedingly difficult to dissect into component cell types. Thus, *Bulla* BRNs provide a unique opportunity to study and identify basic clock constituents. In collaboration with Dr. G. Block, University of Virginia. We have determined that heat shock proteins are involved in temperature-induced resetting of the BRN clock, which is especially interesting in light of recent (unpublished) work of Dr. Joseph Takahashi at Northwestern University showing that heat shock proteins are also elevated when either hamster or chick pineal clocks are reset (or, phase-shifted) by light.

Our third model is the developing circadian pacemaker The gypsy moth testis releases sperm in a circadian fashion. The unique feature of this circadian system is that it is completely operational in vitro, and can also be studied during the course of its development. This enables us to study a circadian system as it is being assembled, which may help to reveal the distinctive components of circadian pacemakers that are difficult to discern in fully developed systems. Finally, the pacemaker has been localized to one of two discrete tissue types, basilar membrane and upper vas deferens. This system is ideal for the study of circadian rhythmicity in that the tissue under study subserves a single function, namely, the production of fertile sperm. In collaboration with Dr. Jadwiga Gieblutowicz, University of Maryland and USDA. We have found about 20 proteins that undergo significant changes, which coincide with the initiation of pacemaker function. Some of these proteins are tissue-specific, and some are rhythmic. Since the reproductive tract is fully differentiated several days before the circadian pacemaker is functional and no other changes are known to occur in this tissue during this stage of development, it is likely that many of the protein changes that coincide with pacemaker maturation are, in fact, pacemaker proteins. It is, however, also possible that some of the protein changes are not related to the pacemaker. Our current experiments have been designed to distinguish between these possibilities and, further, to extend our results not only to protein abundance as measured by silver-stained two-D gels, but also to measure changes in protein synthesis using ^{35}S-methionine. In addition, we have expanded the study to identify rhythmic proteins. Efforts are currently underway to identify the amino acid sequence of the more prominent of the "candidate clock proteins" we have provisionally identified.

F. Development of a Comprehensive Clinical Database from Two-Dimensional Electrophoresis Imaging

High resolution two dimensional electrophoretic investigations of plasma proteins have been much more difficult than those the laboratory performed in CSF primarily because of the higher concentration of proteins in the plasma. A number of the plasma proteins have been

identified by the Anderson's and their colleagues on high resolution 2-D electrophoretograms. This Laboratory continues to update these high resolution 2-dimensional protein maps. Many of the proteins that can be visualized both in plasma and CSF have been studied in normal physiologic states and disease states. We have collected the literature concerning these proteins and their quantitative alterations for over a decade. In collaboration with Dr. Peter Lemkin at the National Cancer Institute, we are using this information to construct a database to help us interpret protein alterations in diseases affecting the CNS. The database may give us some insight into the underlining pathophysiology of some of the CNS diseases of unknown etiology, such as Alzheimer's disease and schizophrenia, that we are currently investigating.

PROPOSED COURSE OF PROJECTS

A. **Induction of APP in Subcortically Lesioned Rat Cortex**

This investigation will be continued in four specific areas (All, as with the initial experiments, in collaboration with Dr. V. Haroutunian of Mount Sinai Department of Psychiatry):

(1) Currently, we are detailing the anatomic regions of the cortex that exhibit the APP induction using in situ hybridization. We will identify first, the cortical areas and layers subsequently, the specific cells, neuronal and/or glial, that respond to each lesion with the induction.

(2) We will determine the precise conditions within the brain that cause induction of APP. To ascertain that the loss of the subcortical source of neurotransmitter is the most direct event responsible for the induction we will treat rat brains with various pharmacological antagonists that will eliminate the biological activity of the neurotransmitter without affecting other synaptic function. We will determine the relationship of other biological factors in the brain such as nerve growth factor and cytokines to further understand the role of extracellular APP in neuronal plasticity. We will determine whether addition of exogenous NGF to the effected brain regions will overcome the induction of APP. Further, because the APP gene contains a promoter region that can be activated by cytokines, we will investigate if cytokines act as the immediate signal to induce the APP.

(3) We will collaborate with Dr. Ron Hart (Rutgers University) who has found that denervation of isolated rabbit superior cervical ganglia will induce the cytokine, IL-6. We will test this same model to determine if APP is similarly induced. Such an observation will give us an *in situ* cellular counterpart of the brain lesions and will associate cytokine activity with APP induction. This ganglion model will facilitate a more precise molecular dissection of the signals leading to APP induction.

(4) We will investigate APP turnover in the lesioned rat brain to understand the lack of senile plaques in the cortex which is overexpressing APP. We will explore the relationship between increased turnover of APP and (1) its phosphorylation state by identifying phosphorylated forms of APP as distinct isoforms on two dimensional gels and (2) generation of potentially amyloidogenic C-terminal fragments that arise from the cleavage of APP at the plasma membrane and resulting secretion of the extracellular domain. We have arranged to test transgenic mice which contain altered forms of the APP gene (including predominant expression of the APP isotype which contains the Kunitz protease inhibitor insert, of which the rat brain usually expresses only small amounts). We hypothesize that the ability of the lesioned cortex to effectively catabolize the overexpressed APP will be disrupted with an altered form of the gene. If this disruption results in the generation of the amyloid peptide, then we may be able to dictate the anatomical location of the production of senile plaques with the appropriate placement of the subcortical lesion.

B. **Abnormal Phosphorylation of Tau to Form A68 in Heat Shocked Neuronal PC12 Cells**

Our results indicate that the altered phosphorylation of tau to form A68 is not sufficient to produce neurofibrillary tangles (NFT), in heat shocked neuronal PC12 cells at least in relatively short term experiments. Therefore, we will extend the time periods for the heat shock treatment to determine whether prolonged stress can cause the PC12 cells to form NFTs. If they do, we will then have a dynamic model with which to study the steps involved in NFT formation. If no NFTs occur, then we will need to elucidate what other pathological events need to occur. Long term stress of neuronal PC12 cells (heat shock or induced by chemical means) will become a model of other neurodegenerative disorders. Understanding the biochemical correlates within the PC12 cells will point to potential cellular pathologies in the injured or aging neuron.

In addition, the abnormal phosphorylation of tau to form A68 will be investigated. Tentatively, we propose that the altered phosphorylation of tau is due to the altered ability of the tau molecule to serve as a substrate for the C kinase. We will confirm that protein kinase C is responsible for the phosphorylation and characterize the differences between the normal phosphorylation sites of tau and the abnormal site(s) in A68. We will determine the role of hsp 72 in protecting against the abnormal phosphorylation. It may be preliminarily hypothesized that heat shock results in an altered confirmation of tau, which if unprotected (as in complexing with hsp 72) will uncover phosphorylation sites normally inaccessible to kinases. To test this possibility, we will assay tau alone, the hsp72:nascent tau complex, and tau within heat shocked cells to determine their susceptibility to phosphorylation by protein kinase C. We will use anti-sense RNA knockout experiments to elucidate the role of hsp in the tau alterations.

C. **Proteins Involved in the Generation and/or Regulation of Circadian Pacemakers**

We are currently attempting to purify the two proteins present in hamsters containing the tau mutation which causes a large altered period of the circadian rhythm. With sufficient purified protein, a partial amino acid sequence will be determined. We will investigate the roles of heat shock proteins in light induced resetting of the clock in the basal retinal neurons of the mollusk, Bulla. Finally, we will attempt to differentiate from among the 20 proteins that significantly change upon developmental initiation of pacemaker function in the gypsy moth testis. Initially, we will determine which of these proteins is newly synthesized with prior incubation with [35S] methionine. We are also identifying rhythmic proteins within this system. The most predominant proteins will be sequenced for identification and characterization.

D. **Association of α 2 FS Haptoglobin and Alzheimer's Disease and Schizophrenia**

In order to understand the role of xFS haptoglobin in AD and schizophrenia, we will conduct an association study. First, we plan to determine whether there is a genetic association between the α 2FS form of haptoglobin, AD, and schizophrenia. These studies will involve analysis of protein isoforms as well as the use of PCR markers located in the haptoglobin gene. Second, we will correlate the quantitative levels of xFS haptoglobin with the course of the disease from autemortem candidates for AD (such as siblings of AD patients) to end stages of the disease.

E Proteins Involved in the Regulation of Mammalian Hibernation

No definitive therapy for human stroke is currently available, largely due to our poor understanding of the mechanisms underlying the progressive neurological damage that occurs in the early hours of brain ischemia. This study (in collaboration with Drs. J. Hallenbeck and K. Frerichs; Drs. H. Gainer and H. Jaffe; NINDS) is designed to provide an unbiased approach towards identifying changes associated with hypoxia and ischemia. We will investigate altered gene expression that occurs during hibernation. Hibernation is a natural mechanism used by a variety of species to adapt to a hypothermic, hypoxic, and hypometabolic state to minimize energy expenditure in response to seasonal cold and shortages of food and/or water.

We will identify protein changes that occur as part of natural hibernation in ground squirrels, by comparing proteins in samples collected from animals in both awake and hibernating states. CSF and plasma samples are compared within individual animals that have been sampled during both states. Thus far, we have run two-dimensional gels on brain, CSF, and plasma samples from these animals. Our preliminary analysis of plasma samples has indicated about five different proteins that are altered during hibernation.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 00935-25 LBG

PERIOD COVERED
October 1, 1991 to September 30, 1992

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	LBG,NIMH
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	L. Mitchell	Staff Fellow	LBG, NIMH
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COOPERATING UNITS *(if any)*
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LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Biochemical Genetics Section

INSTITUTE AND LOCATION
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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.25	1.25	1

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

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*
There have been recent numerous reports of accumulations of mutations in the mitochondrial genome in a variety of human diseases and with age. It has been proposed that these mutations may be due in part to the mitochondrial oxidative pathways which provide most of the cell's energy in the form of ATP but generate potentially hazardous free radicals in the process. To examine for the occurrence of mitochondrial mutations in the central nervous system, we have amplified and sequenced regions of the mitochondrial genome from CNS tissue obtained from individuals of varying ages. In one study, we sequenced 32,000 base pairs of mitochondrial DNA cloned from retinal tissue from a 71 year old individual. This approach revealed only one heterotypic mutation. A second study evaluated mitochondrial DNA isolated from human brain tissue by widely spaced PCR analysis. This technique employs distant PCR primers and PCR conditions optimized to amplify DNA harboring large scale deletions. Large mitochondrial DNA deletions were detected in increasing amounts in individuals over 27 years of age. The laboratory plans to investigate the significance of these deletion mutations and their relationship to the aging process and disease states.

PHS 6040 (Rev. 5/92)

OBJECTIVES:

The mitochondrial genome has been shown to have a high evolutionary mutation rate (5 to 10 times that of the single copy nuclear genes). This high mutation rate may be explained by the lack of both replicative and post-replicative DNA repair mechanisms in the mitochondria. Due to the apparent lack of repair mechanisms, the human mitochondrial genome may serve as a good indicator for the accumulation of somatic mutational events in post-mitotic tissues. Studies of somatic mutational events in the mitochondrial genome may provide insights into pathophysiological processes.

Recent studies have discovered that mutations in the mitochondrial DNA may play a causal role in a variety of human diseases. It appears that key base substitutions or major deletions may result in disruption of oxidative phosphorylation and consequently mitochondrial dysfunction. Given the high energy demands of the human brain, the probability exists that defects in the mitochondrial genome could play a role in the neuronal degeneration observed in disorders such as Parkinson's disease, Alzheimer's disease, Huntington's Chorea or the normal aging process.

This laboratory's objectives are to evaluate the mitochondrial DNA of human brain tissue from patients with neurological disorders as well a normally aged individuals for the presence of mutational events which may play a role in the degenerative processes observed in these diseases and in normal aging. Previously, we examined human retinal tissue from a 71 year old to evaluate the mutational damage acquired over the life span of an individual. No evidence for age-related accumulations of mutations in retinal mitochondrial DNA was found when 32,328 basepairs from 83 different clones were sequenced. We had expected to find a much higher accumulation of mutations based upon the reported error rate for the DNA polymerase that replicates the mitochondrial genome and the lack of DNA repair mechanisms.

Additional studies have been performed using PCR analysis to evaluate the presence of deletion mutations in association with neurological disease and the normal aging process. Standard PCR amplifications were employed to evaluate the occurrence of mitochondrial DNA additions or deletions within mitochondrial genes encoding the subunits of Complexes I and IV. A second set of PCR amplifications using widely spaced primer PCR technology were aimed at detecting large deletions which spanned multiple genes.

METHODOLOGY:

These studies were conducted using DNA purification procedures, including centrifugation, chromatography and electrophoretic techniques. Oligonucleotide primers were synthesized on a DNA synthesizer. The laboratory utilizes DNA cloning, DNA sequencing by the Sanger method, restriction endonuclease analysis, DNA hybridization, agarose and polyacrylamide gel electrophoresis and Polymerase Chain Reaction (PCR) methodologies. Several PCR strategies were employed including widely spaced PCR analysis and asymmetric PCR amplification. DNA is detected by ethidium bromide staining, hybridization with specific probes and autoradiography.

MAJOR FINDINGS:

Intra-genic studies failed to detect any additions or deletions within the amplified portions of the genes encoding subunits of Complexes I and IV. However, several polymorphic variants were detected at restriction endonuclease cleavage sites and were subsequently sequenced. Both of the base substitutions sequenced were G-A transitions at the third nucleotide of the triplet codon resulting in "silent" mutations. Although these mutations resulted in a loss of a restriction enzyme site, they should not alter the translation product of the gene.

Inter-genic studies employed widely-spaced PCR analysis to evaluate the presence of large deletions which spanned multiple genes. A large deletion of approximately 5,000 base pairs was detected in all normal and disease brain tissue from individuals 27 years old or older. The intensity of the amplified band indicative of the deletion increased with the increasing age of the individual. In addition, a limited evaluation of various brain tissues indicates that there may be quantitative differences in the deleted genomes dependent upon the brain region. Additional studies will evaluate the possibility that this may be related to the varying metabolic rates of different regions of the brain.

The laboratory has shown that there is an association between deleted brain mitochondrial DNA species and the aging process. Whether these mutant molecules play a causitive role in the generalized degeneration accompanying aging or are a relatively harmless accumulation of free radical damage over the lifespan of an individual can not be determined at this time. Further evaluation of their presence in additional tissues and their association with mitochondrial respiratory enzyme activity levels may shed some light on whether the accumulation of deleted mitochondrial genomes which have been shown to be pathogenic in disorders such as Kearns-Sayre syndrome, chronic progressive external ophthalmoplegia and related mitochondria myopathies are an important contributor to the progress of age-related degenerative diseases and the aging process in general.

PROPOSED COURSE OF RESEARCH:

The laboratory will continue to evaluate the presence of deleted mitochondrial DNA species both in aged individuals and in patients with neurological disorders. A variety of brain regions will be evaluated by the widely spaced PCR technology to determine if there is an association between metabolic rate of a specific tissue and the level of deleted molecules. The presence of the mutant genomes will be compared with the mitochondrial respiratory enzyme activity levels in different age groups and in disease states.

The specific deletion which has been associated with the aging process will be cloned and sequenced. Analysis of the deletion juncture may provide information for the construction of a model for the creation of these deleted genomes and may elucidate their role, if any, in the normal and aged individual. DNA sequencing will allow for the comparison of this age associated deletion and previously reported deletions observed in mitochondrial myopathies and Kearns-Sayre syndrome.

PUBLICATIONS:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 01559-11 LBG

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neuropeptide FF (FLFQPQRF-NH_2) in the Brain and Spinal Cord: Function and Distribution

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

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	J. Zhu	Visiting Fellow	LBG, NIMH
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COOPERATING UNITS *(if any)*

Dr. R. Rothman, NIDA Addiction Research Center, Baltimore, MD; Dr. A. Beckman, State *University of California, CA.*

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Laboratory of Biochemical Genetics

SECTION
Neuropeptides

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeth's Hospital, Washington, DC 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.6	0.6	1.0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects _(b) Human tissues _x(c) Neither
_(a1) Minors
_(a2) Interviews

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

Neuropeptide FF (FLFQPQRF-NH_2) was originally isolated from the bovine brain and found to have morphine modulating activity. Recently there are studies suggesting the involvement of neuropeptide FF (NPFF) in morphine abstinence and tolerance. In this study the role of endogenous NPFF in opiate dependence was further assessed using NPFF antibody as a specific antagonist. Rats, which were rendered dependent to opiate by morphine pellets, simultaneously received icv infusions of anti-NPFF IgG or control IgG. Naloxone induced abstinence signs were significantly lower in the group treated with anti-NPFF IgG than the group treated with control IgG. NPFF is highly localized in nerve terminals in the posterior pituitary. Superfusion of posterior lobes of rat pituitaries demonstrated that NPFF can be released from the pituitary by depolarizing concentration of KCl. Using this posterior pituitary as a model, we have found that naloxone can cause a significant release of NPFF from the pituitary of the rat pretreated with morphine. The amounts of NPFF released seem to increase with increasing dose and duration of the morphine treatment. The results strongly indicate that release of NPFF may participate in the naloxone precipitated opiate withdrawal. In order to further study the possible role of NPFF in opiate withdrawal and tolerance, work was initiated to attempt to isolate a specific cDNA clone for NPFF precursor protein. We are also currently isolating other NPFF-like peptides from adrenal and pancreatic glands with the aim of characterizing them both chemically and biologically.

PHS 6040 (Rev. 5/92)

PROJECT DESCRIPTION

Neuropeptide FF (FLFQPQRF—NH_2), NPFF was originally isolated from the bovine brain in our laboratory and accumulating evidence to date suggests that NPFF may have a modulatory role in some endogenous and exogenous opiate mediated effects. In order to further understand the functional role of NPFF, this study was directed to 1) possible role of NPFF in opiate dependence (collaboration with Dr. R. Rothman, NIDA Addiction Research Center and Dr. A. Beckman, California State University), 2) NPFF in pituitary and 3) NPFF-like peptides in peripheral tissues. We, together with Drs. Steve Zullo and Carl R. Merril, have also initiated a project for isolation of NPFF precursor (Prepro-NPFF) cDNA clone.

Recently, there are studies suggesting the involvement of NPFF in morphine abstinence syndrome. In this study, the role of endogenous NPFF in morphine dependence was further assessed using IgG prepared from the antiserum raised against NPFF as NPFF antagonist. Rats were rendered dependent to morphine by implantation of 2 morphine pellets on day 1, and 4 pellets on day 2. Simultaneously these rats also received anti-NPFF IgG or control IgG infused into the lateral ventricle. On day 5 of the protocol, naloxone was injected icv and dependence was determined. Opiate abstinence signs (including body weight loss, wet dog shakes, chewing and salivation) induced by naloxine were significantly lower in the group treated with anti-NPFF IgG than the group treated with control IgG. The results further support the hypothesis that NPFF is involved in morphine abstinence syndrome.

We were also exploring the role of NPFF in opiate dependence in hibernating ground squirrels. Beckman et al. have previously reported that physical dependence on morphine occurs in a typical fashion during the active state of the mammalian hibernator Citellus lateralis (ground squirrel), but does not occur when morphine exposure is confined to the hibernating state. This observation indicates that the ground squirrel may be an useful model to study the possible involvement of NPFF in opiate dependence. The experimental results on the ground squirrel to date indicate that authentic NPFF exists in ground squirrel CNS and it is unevenly distributed in the brain. The possible effect of hibernation and morphine treatments on this NPFF distribution is currently being studied.

The highest concentration of NPFF is found in the posterior pituitary gland. Although the modulatory action of opiates on posterior pituitary hormone secretion has been extensively investigated, the role of NPFF in pituitary function still remains unclear. In order to explore the functional role of NPFF in the hypothalamo-neurohypophyseal system, we have decided to study the release of NPFF from the rat pituitary and the effect of opiates on this NPFF release. The experimental results to date indicate that NPFF can be released by a depolarizing concentration of KCl and this release is calcium dependent. This result is in keeping with the ultrastructural studies of other investigators which indicate NPFF immunoreactive material is localized to nerve terminals in the rat posterior pituitary. In exploring the possible interactions between NPFF and opiates, we have observed that naloxone can cause a significant release of NPFF from the posterior pituitary of rats pre-treated with morphine. The preliminary experiment has also shown that this NPFF release from the posterior pituitary is especially pronounced when rats were treated repeatedly with increasing doses of morphine for up to 4 days. We plan to further assess the subtypes of opiate receptors involved in this NPFF release especially the kappa opioid receptor which is known to be highly localized in e posterior pituitary.

One of the paradoxal clinical features of opiate dependence after chronic opiate administration is that low doses of opioid antagonist, naloxone, can induce autonomic and psychic withdrawal symptoms. The cellular mechanism underlying this naloxone-triggered withdrawal has been intensively investigated but still remains unclear. We have observed that naloxone can induce a release of NPFF from pituitaries and also from spinal cords (see the annual report # Z01MH 02505-02 LBBB) of rats pretreated with morphine. Furthermore, it has been reported that NPFF can precipitate an abstinence syndrome in morphine dependent rats. These observations taken together imply that NPFF may provide an additional interesting system to study the naloxone triggered opiate withdrawal and opiate dependence. In order to pursue further the

possible role of NPFF in opiate dependence and tolerance, a study (together with Drs. S. Zullo and Carl R. Merril) was initiated to prepare a cDNA library in order to isolate a specific cDNA clone for the NPFF precursor protein (prepro NPFF). In the rat hypothalamus, we have previously demonstrated the existence of NPFF containing cell bodies in supraoptic nuclei and also in periventricular area between the dorsomedial and ventromedial nuclei. These hypothalamic regions containing NPFF positive neurons have now been punched and RNA extracted. We are in the process of preparing a cDNA library from this RNA, which may be enriched with NPFF precursor protein mRNA, with the goal of isolating an NPFF specific cDNA clone.

In this study the possible presence of NPFF in periphery was also examined. NPFF-like immunoreactive material was detected in rat adrenal and pancreatic glands. Three NPFF immunoreactive peptides were detected and partially purified. Chromatographic studies indicate that they are different from authentic NPFF. Since there are studies indicating that 1) NPFF can exerts a potent action in the pancreatic insulin secretion and 2) NPFF receptors exists in the rat adrenal glands, we plan to further purify these NPFF immunoreactive peptides for both biological and chemical characterizations.

SIGNIFICANCE TO THE BIOMEDICAL RESEARCH

The present study suggest that the endogenous neuropeptide FF (FLFQPQRF-NH_2) may be involved in the supersensitivity that develops to the excitatory effect of opioid antagonist in animals receiving chronic opiates.

PROPOSED COURSE OF STUDY

We plan to continue 1) the study on the possible interactions between opiates and neuropeptide FF, 2) the isolation of a neuropeptide FF precursor-protein specific cDNA clone and 3) the purification of NPFF-like peptides from adrenal and pancreatic glands for biological and chemical characterization.

PUBLICATIONS

Lake JR, Hammond MV, Shaddox RC, Hunsicker LM, Yang H-YT, Malin DH. IgG from neuropeptide FF antiserum reverses morphine tolerance in the rat, Neuroscience Letters 1991; 29-32.

Majane EA, Yang H-YT. Mammalian FMRF-NH_2-like peptides in rat pituitary: decrease by osmotic stimulus, Peptides 1991;12:1303-1308.

Rothman RB, Xu H, Yang H-YT, Long JB. Anti-opioid peptides in morphine tolerance and dependence: focus on NPFF, Neurobiology of Opiates CRC Press Inc In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02506-03 LBG

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Secretion of Neuropeptide (FLFQPQRF-NH_2) Endogenous Antiopioid Peptides

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

PI:	J. Zhu	Visiting Fellow	LBG, NIMH
Other:	H.-Y.T. Yang	Section Chief	LBG, NIMH

COOPERATING UNITS *(if any)*
Department of Pharmacology & Toxicology, Queen's University, Kingston, Ontario Canada (K. Jhamandas).

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Neuropeptides

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeth's Hospital, Washington, DC 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

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

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

NeuropeptideFF (FLFQPQRF-NH_2) is an endogenous neuropeptide with morphine modulating activity. Neuropeptide FF (NPFF) immunoreactive nerve terminals and NPFF receptors were found to be highly localized in the superficial laminae of rat dorsal spinal cords. These observations strongly suggest a role for NPFF in the spinal cord function. In order to explore the functional role of NPFF, the secretion of NPFF from the rat spinal cord was studied. Furthermore, the possible effects of opiates on this NPFF release were also investigated. Substance P caused a release of NPFF from the spinal cord in a calcium dependent manner. Further studies with other substance P related peptides used as secretagogues indicated that a specific substance P receptor (SP-N) is involved in NPFF secretion from the spinal cord. In view of the role of substance P in sensory transmission, the results of this study strongly suggest a modulatory role for NPFF in antinociception. The study on the effect of morphine on NPFF efflux has demonstrated that naloxone can induce a release of NPFF from the spinal cord of rats pretreated with morphine. In contrast, naloxone was found to have nearly no effec on the basal efflux of NPFF. Other investigators have previously reported that NPFF administered ICV (third ventricle) into morphine-dependent rats can precipitate an opiate abstinence syndrome. Thus the results of this study strongly indicate that NPFF may be involved in naloxone precipitated abstinence syndrome in morphine dependent animals.

PHS 6040 (Rev. 5/92)

Project Description

Neuropeptide FF (FLFQPQRF-NH_2) was originally isolated from the bovine brain and found to have modulatory effects on some opiate mediated responses. Neuropeptide FF (NPFF) is unevenly distributed in the central nervous system with the highest concentration in the spinal cord. In this project, the release of NPFF from rat spinal cords was investigated with the aim of exploring the functional role of NPFF in the spinal cord. Using an in vitro superfusion of an isolated rat spinal cord, we have previously demonstrated for the first time that NPFF can be released in a calcium dependent manner by a depolarizing concentration of KCl or substance P. In this study 1) substance P receptor subtypes involved in the release of NPFF and 2) possible interactions between opiate and NPFF were investigated.

To assess possible substance P receptor subtypes involved in the release of NPFF, in addition to substance P (1-11), other substance P related peptides were also tested as secretagogues. It was found that release of NPFF can also be elicited by substance P(1-7), the N-terminal substance P fragment, but not by [$pGlu^5$, $Me\text{-}phe^8$, Sar^9]-substance P(5-11), the stable analogue of a C-terminal substance P fragment. Substance K, which shares a common C-terminal sequence with substance P(1-11) also failed to elicit NPFF release from the spinal cord. The results strongly suggest that the specific substance P receptor (SP-N), which is recognized by both substance P(1-11) and substance P(1-7), rather than the tachykinin receptor, is involved in NPFF secretion from the spinal cord. Other neurotransmitters such as serotonin, norepinephrine, NMDA and glutamate were found to exert no effect on the efflux of NPFF from the spinal cord. In view of the role of substance P(1-11) and substance P(1-7) in sensory transmission, the results strongly suggest a modulatory role for NPFF in antinociception in the spinal cord.

Possible interactions between opiate and NPFF were investigated by looking for the effects of opiates on the release of NPFF from rat spinal cords. It was found that naloxone caused a significant release of NPFF from the spinal cords of rats pretreated with morphine. In contrast, naloxone exerts a very slight increase or no effect on the basal efflux of NPFF from the spinal cords of control rats. Furthermore, amounts of NPFF released seem to increase with the frequency of the morphine treatments. We are currently in the process of establishing the specificity of this naloxone effect. The results of this study to date appear to suggest an important role for NPFF in the development of morphine dependence.

Previously we observed that release of NPFF induced by depolarizing concentration of KCl could be inhibited by the addition of morphine to the perfusion medium. In this study, possible opiate receptor subtypes involved in this inhibitory activity of morphine was investigated. The preliminary results indicate that the kappa opioid receptor agonist is highly effective in inhibiting the evoked release of NPFF from the spinal cord. We are still in the process of testing other opioid receptor ligands.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

The results of this study indicate that Neuropeptide FF (FLFQPQRF-NH_2) may play a role in opioid dependence and further study on this endogenous peptide may lead to a better understanding on the mechanism of opiate dependence.

PROPOSED COURSE OF STUDY

We plan to extend this study to assess the role of neuropeptide FF in methadone dependence. Experiments will also be designed to assess the role of neuropeptide FF in opioid tolerance.

PUBLICATION

Zhu J, Jhamandas K, Yang H-YT. Release of neuropeptide FF (FLFQPQRF-NH_2) from rat spinal cord, Brain Research In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02505 03 LBG

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Trophic Material in Neural Tissue

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

PI:	J. Zhu	Visiting Fellow	LBG, NIMH
Other:	H.-Y.T. Yang	Section Chief	LBG, NIMH

COOPERATING UNITS *(if any)*
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LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Neuropeptides

INSTITUTE AND LOCATION
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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0

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

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

During the development of a primary culture of chromaffin cells from adult rat adrenals, neurite out-growth from chromaffin cells was sometimes observed. Subsequently we collected the adrenal medullary culture media in which chromaffin cell neurite outgrowth was observed and found that it stimulated neurite out-growth from cultured adrenal chromaffin cells and also from PC-12 cells. The experimental results to date indicate that this neurite out-growth activity is a heat labile protein but is distinct from NGF, aFGF, bFGF, EGF and IGF-I. Recently, it has been reported that various protease inhibitors are capable of initiating neurite-outgrowth from various cells. Because of this, various protease inhibitors, α_2-macroglobulin, soybean trypsin inhibitor, α_1-antitrypsin, aprotinin, leupeptin and antipain were examined and found to exert no neurite-outgrowth activity on PC-12 cells. Our previous experiments also indicate that adrenal medullary non-chromaffin cells may release this neurite-outgrowth activity into the culture media during culture. In this study, large quantities of adrenal medullary cells were prepared for extractions of the neurite out-growth promoting factor(s) and some extracts were found to be active in initiating neurite-outgrowth from PC-12 cells. We plan to establish an optimal extraction condition and then to isolate this neurite-outgrowth factor(s) for further characterization.

PHS 6040 (Rev. 5/92)

PROJECT DESCRIPTION

During the development of a primary culture of adrenal chromaffin cells from adult rats, we observed that, under the conditions used, chromaffin cells survived a very long term of culture (6 month or longer). Furthermore, spontaneous neurite-outgrowth from chromaffin cells was sometimes observed. Subsequently, the adrenal medullarycell culture media in which chromaffin cells extended neurites were collected and the neurite-outgrowth promoting activity was characterized. The conditioned medium was found to promote neurite out-growth from primary cultures of adrenal chromaffin cells and also from PC-12 cells. Using neurite-outgrowth from PC-12 cells as a bioassay, we found that the neurite-outgrowth activity is due to a heat labile protein which is different from NGF, aFGF, bFGF, EGFD and IGF-I. Recently, there are studies indicating that some protease inhibitor are capable of initiating neurite-outgrowth from various types of cells. Because of this, the effects of various protease inhibitors including α_2-macroglobulin, soybean trypsin inhibitor, α_1-antitrypsin, aprotinin, leupeptin and antipain, on PC-12 cells were examined. All of the protease inhibitors (at 100 μg/ml) failed to initiate neurite-outgrowth from PC-12 cells under our culture conditions. In order to further characterize this neurite-outgrowth activity, we decided first to search for a better source than the conditioned medium for this factor(s). Our previous experimental results strongly suggest that the neurite-outgrowth promoting activity is released from non-chromaffin adrenal medullary cells into the culture medium. In this study, large quantities of adrenal medullary non-chromaffin cells were prepared and extracted. Some adrenal medullary cell extracts were found to exhibit neurite-outgrowth activity when added to the PC-12 cell culture. We are in the process of establishing an optimal extraction protocol in order to isolate this neurite-outgrowth promoting factor for further characterization.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

While the exact nature of this neurite-outgrowth promoting factor awaits isolation and characterization, this factor may be an interesting molecule to be considered in adrenal tissue transplantations in experimental Parkinsonian models.

PROPOSED COURSE OF STUDY

We plan to search for a better source for this neurite-outgrowth factor and then to isolate this factor for characterization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02571-02 LBG

PERIOD COVERED
October 1, 1991 to September 30, 1992

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

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

PI:	M.H. Polymeropoulos	Visiting Scientist	LBG,NIMH
	C.R. Merril	Chief, Biochemical Genetics	LBG, NIMH
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	J. Rubenstein	Biologist	LBG,NIMH
	R. Torres	Grad. Student	LBG, NIMH
	A.. Glodek	Computer Programmer	LBG, NIMH
	C. Venter	Lab Chief	NINDS
	L. Delisi	Assoc. Prof.	SUNY at Stonybrook, NY
	T. Crow	Head Div. Psych.	Medical Research Council
	J. Sikela	Assistant Prof.	Univ. of Colorado
	J.L. Weber	Sen. Scientist	MMRF, Wisconsin
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COOPERATING UNITS *(if any)*
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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

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_(a1) Minors
_(a2) Interviews

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

The Laboratory has continued and expanded on the efforts towards the mapping of the human genome. The specific aims are:

1. Development and characterization of highly informative microsatellite repeat polymorphic markers.

2. Development of sequence tagged sites and chromosomal assignment of brain expressed genes.

3. Genetic linkage studies of Schizophrenia and Bipolar disorder, using the microsatellite repeat polymorphisms.

PHS 6040 (Rev. 5/92)

OBJECTIVES

1. Development and characterization of microsatellite repeat polymorphic markers.

We will continue the development of microsatellite repeat markers. Emphasis will be placed on the isolation and sequencing of 100 microsatellite polymorphic sequences from human brain cDNA libraries. Estimation of informativeness will be performed, and chromosomal assignments will be determined.

2. Genetic mapping of polymorphic markers.

The position of the microsatellite polymorphisms on the genetic map will be determined by studying the segregation of the alleles in the 40 CEPH reference families. All of the previously developed markers will be placed tentatively on the map by studying the allelic inheritance in 5 families. We plan to request additional funding support from the NIH Human Genome Office for the refined mapping of all the markers developed in the Laboratory.

3. Physical mapping of brain expressed genes.

We will continue the mapping of brain expressed sequence tags on the human chromosomes by analysis of somatic cell and radiation hybrids. We plan to augment the mapping effort by mapping human expressed sequences on a mouse backcross and inferring the human localization by syntenic homology. This project is being funded by a grant from the Department of Energy.

4. Genetic linkage mapping of Schizophrenia.

We will continue the genetic linkage mapping of 34 small families with Schizophrenia using microsatellite repeat polymorphisms. We plan to type 200 markers in total and perform linkage analysis under different numbers of models of transmission.

5. Genetic linkage mapping of Bipolar disorder.

In collaboration with intramural and extramural laboratories we will continue the mapping of Bipolar susceptibility gene or genes, in a large Canadian pedigree. In this effort we plan to type 60 microsatellite markers scattered on 11 human chromosomes.

METHODS

Somatic Cell Hybrids

The human-rodent somatic cell hybrid lines used in this mapping protocol were, the Bios PCRABLE DNA, from the Bios Corp. New Haven, CT and the NIGMS Human/rodent somatic cell hybrid mapping panel #1 from NIGMS, Camden, NJ. The BIOS panel is a human-hamster somatic cell hybrid panel. The NIGMS panel is primarily based on a mouse background with the exception of one of the lines in this panel, containing human chromosome 9 as its only human chromosome component, that is a human-hamster somatic cell hybrid.
Sequence analysis cDNA sequences of approximately 350 base pairs (BP), were obtained from the partial sequencing of brain cDNA clones. We analyzed these sequences to define short 80-100 b.p. fragments which would have the least chance of containing an intron in the genomic DNA. Pairs of oligonucleotides were designed for PCR amplification. To assist in this effort we have developed the computer program intron based on the following assumptions : (1) Introns are

genomic sequences with an average length of 600 base pairs which interrupt the coding and non-coding sequences of genes. (2) There are two consensus sequences for the intron-exon splice junctions: one for the 5' and another for the 3' end of the junction. From analysis of these sequences, it appears that the dinucleotide GG constructed from the most distal and the most proximal end of the exonic sequence is conserved in approximately 80% of the intron-exon junctions. (3) It has been shown that for approximately 90% of the human genes studied the 3' untranslated region of a mRNA is not interrupted by introns in the genomic DNA, so that a GG dinucleotide surrounded by stop codons in all three reading frames most likely will not represent an intron-exon junction. The program evaluates the likelihood that a given GG or CC dinucleotide represents a former exon-intron boundary. The input and output file are in ASCII text format compatible with the standard used by the Intelligenetics Suite and other sequence analysis programs. This computer program is written in C programming language and currently runs on a SUN Workstation.

MAJOR FINDINGS

Chromosomal Assignment of cDNAs

We have established chromosomal assignment for 323 brain derived expressed ESTs using polymerase chain reaction PCR and human-rodent somatic cell hybrids. We have developed a procedure for the rapid chromosomal assignment of these ESTs: EST sequences are first analyzed by the computer program INTRON to determine regions likely not to be interrupted by introns in the genomic DNA. A pair of oligonucleotide primers is then designed to amplify this region by the PCR using DNA template from human-rodent somatic cell hybrid chromosomal panels. The chromosomal assignment of the EST is determined by studying the segregation of the amplified products in these panels.

The designed oligonucleotide primers amplified the desired product in 81% of the sequences analyzed. Subsequently, chromosomal assignment could be established for 88% of the amplified ESTs. Interference from the rodent background accounted for 8% of the failures to assign, 1% was contributed by a total of three genes with multiple gene members, and 3% could not be mapped because of various reasons such as high human background or weak signal.

Sublocalization of cDNAs

We have established collaborative efforts with several groups for the sublocalization of the cDNAs. Sublocalizations are being pursued for chromosomes 1, 4, 6, 7, 8, 9, 10, 13, 15, 17, 21, and X. Specifically we have collected radiation hybrids for chromosomes 7, 10, and 9, as well as deletion hybrids for chromosomes 8 and 9. In this effort we continue to characterize the chromosomal content of the hybrids, by typing microsatellite repeat markers with known sublocalizations.

Mouse Backcross

With the construction of a genetic map of the mouse along with the delineation of the areas of syntenic homology between the human and mouse, it is possible to infer the localization of human genes by studying the segregation of a human cDNA on the mouse chromosomes. In order to pursue this mapping approach, we have, in collaboration with Dr. Beverly Mock of NCI, developed a mouse interspecific backcross between Balb/c and M. spretus. The base map will be constructed with (AC)n repeat microsatellites developed at MIT.

Polymorphic cDNAs

We have determined that 1-2% of the clones in any given cDNA library contain (AC)n repeat sequences in their 3' untranslated regions. We have isolated 130 cDNA clones containing (AC)n repeat blocks and have sequenced 20 to determine the length of the repeat and the flanking unique sequences. Under the hybridization conditions used the length of the repeat block ranged from 16-20 repeat units. PCR assays were developed for these sequences and the PIC value was estimated from unrelated individuals. The average PIC value for these polymorphic markers was 0.60. Sublocalization of these markers was done by linkage analysis in the CEPH families.

Database

We have developed a cDNA mapping information database based on the Sybase software system. This database is structured so that one part will function as the Labratory's working database while another part will be accessible to the public via an FTP anonymous account. The database includes information on clone name, clone sequence, sequencing primer, PCR primers, chromosomal localization, as well as information on rodent products, multigene families, and polymorphic data.

The above project was supported by a Department of Energy Interagency Agreement initiated on 11/1/91 at a current annual funding level of $203,000 per year.

Mapping of Polymphic Markers

Microsatellite Repeat Markers

We have continued the isolation of dinucleotide repeat markers from human phage and cosmid genomic libraries. This effort has been facilitated by developing a strategy for the isolation of the most informative markers by selecting the ones with the stronger hybridization signals. Since our library clones have large inserts we can select from a large pool of positive clones so that we can access the most informative much faster. Sequencing of large inserts poses technical difficulties since the position of the repeat within the clone is unknown. We have developed a strategy to directly sequence the repeat flanking sequences, by using sequencing primers anchoring at the repeat site.

We have determined rules that predetermine the informativeness of tri- and tetranucleotide repeat polymorphisms based on the size of the repeat sequence. To date, we have isolated, characterized, and mapped 57 polymorphic markers, which include 15 based on tri and tetranucleotide repeat sequences.

In order to facilitate the mapping of these markers on the human genome we have developed a computer program that studies the position of the meiotic breakpoints and proceeds to place any given marker by attempting to minimize the number of meiotic breakpoints on a set map. This program operates on the SUN Workstation systems and is compatible with output of commonly used genetic databases. We have collaboratively used this program in the mapping of chromosome 10 and 6 microsatellite polymorphisms.

Disease Gene Mapping

Schizophrenia

Our global genome scanning effort continues with 50 Schizophrenic families from the U.S. and the U.K. We have to date typed 90 microsatellite markers scattered on the human chromosomes. We have developed a comprehensive two-point exclusion map of human chromosome 5 and we will soon complete a similar map for human chromosomes 2 and 6.

In a parallel effort set to examine the implication of a genetic event which could explain the discordance of Monozygotic (MZ) twins with Schizophrenia, we have scanned the genome with 90 microsatellite polymorphic markers, in five sets of MZ twins. This study failed to identify any genetic discordance, adding to the argument that factors other than genetic may account for this discordance.

Bipolar Disorder

We have completed the typing of 88 markers in the Old Order Amish pedigree in collaboration with intramural and extramural laboratories. We have also assumed responsibility for typing markers on 11 chromosomes in a large Canadian pedigree segregating for Bipolar disorder. In this effort we have completed the genotyping of 40 markers.

Collaborative Gene Mapping Efforts

In the collaboration with intramural and extramural laboratories we have assisted in the mapping of the genes responsible for Treacher-Collins and Ushers Type II syndromes. We are currently involved in a linkage study for the Neimman-Pick type C syndrome.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF ICD

With the advances in the methodology used in the study of the genetic information, it has become feasible to approach the exploration of whole genomes. A first step in the study of the organization of the human genome is the development of maps of the human chromosomes. For many decades the only available complete maps of the human genome have been the cytogenetic maps where individual human chromosomes are identified based on their size and banding pattern. With the conception of the idea to create maps based on the study of meiotic recombination a new era in mapping of the human genome had emerged. The use of the RFLP markers as tools to study genetic recombination and inheritance resulted in the creation of a set of maps for all the human chromosomes. However, the lack of informativeness of these markers seemed to prohibit the development of high resolution maps, since the number of meiosis studied and markers used would have to be very large. The recent discovery of the microsatellite repeat markers seemed to overcome the problem of informativeness, and the methods of genotyping became very efficient.

Our Laboratory is invested in the isolation, characterization, and mapping of this class of microsatellite repeat markers. These genetic markers are based on the variation of the number of repeat units within a repeat block. Based on the size of the repeat unit these markers are called dinucleotide, trinucleotide, tetranucleotide, etc., repeat markers. The most abundant class of these markers is the (AC)n type, numbering approximately 10,000 informative markers in the human genome. The trinucleotide and tetranucleotide markers are far less abundant but still quite useful. Specifically, tri- and tetranucleotide repeat markers are more easily resolved than the dinucleotide ones, making scoring while genotyping easier and errors less common. Furthermore, the recent discovery that human disease gene defects were based on the variation of the number of repeat units of trinucleotide repeat sequences,

makes the study of such sequences imperative. Our laboratory has established the rules to determine which tri-and tetranucleotide repeat sequences will produce informative markers and has developed techniques to isolate and characterize these repeat sequences from human genomic libraries.

Although genetic maps orient us in the genome, construction of physical maps gives us access to the actual DNA sequences. The issue of sequencing the human is still being debated in attempts to judge the cost effectiveness and correct timing. However, a sequencing effort targeting the expressed genes is well underway with several thousand genes already sequenced. Our Laboratory has developed methods to efficiently map these expressed sequence tags onto the human chromosomes. It has been estimated that approximately 100,000 genes are expressed in the human body with 30,000 of them expressed in the human brain alone. Our effort involves the development of highly transportable mapping assays, chromosomal assignment of cDNAs and sublocalization. In order to tie together physical, expression and genetic maps we have concentrated our efforts into the identification of microsatellite repeat sequences within the cDNAs and subsequently map them using the CEPH families and genetic linkage analysis.

Development of these comprehensive physical, expression and genetic maps of the human genome provides us with unique tools for the genetic study of human disease. Our Laboratory has targeted two psychiatric syndromes, namely Schizophrenia and Bipolar disorder. The initial excitement over the achievment of linkage of DNA markers to both Schizophrenia and Bipolar illness has subsided along with the initial positive reports. It has become apparent that global genomic scans with polymorphic markers in large number of individuals will be necessary for the genetic linkage analysis study of these disorders. Such an effort is now feasible with the use of the PCR typable highly informative microsatellite repeat markers. Our Laboratory has undertaken a global search for the identification of gene or genes involved in the pathogenesis of these disorders, by studying the linkage association of anonymous DNA markers with the phenotype in families segregating for these disorders.

The complicated task for the development of maps of the human genome requires the development and implementation of efficient methods of analysis. Our Laboratory has developed a number of algorithms and computer programs to facilitate this analysis. We have also placed emphasis in the timely release and facilitation of transfer of data to other laboratories, and we have developed databases that can be accessed on our anonymous account via file transfer programs.

PUBLICATIONS

Polymeropoulos MH, Xiao H, Merril CR. The human D5 dopamine receptor (DRD5) maps on chromosome 4, Genomics 1991;11:777-778.

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Polymeropoulos MH, Rath DS, Xiao H, Merril CR. Tetranucleotide repeat polymorphism at the human c-fes/fps proto-oncogene (FES), Nucl Acids Res 1991;19(14):4018.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 MH 02572-02 LBG

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of Receptors for FMRFamide-Related Neuropeptides

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

PI:	Dr. Kemal Payza	Staff Fellow	LBG, NIMH
Other:	Dr. Candan Akar	Visiting Fellow	LBG, NIMH
	Dr. H.-Y. T. Yang	Section Chief	LBG, NIMH

COOPERATING UNITS *(if any)*
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Dr. D. H. Malin, University of Houston-Clear Lake, Houston, Texas
Dr. Kevin Burgess, Rice University, Houston, Texas

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Neuropeptides

INSTITUTE AND LOCATION
NIMH Neuroscience Center at St. Elizabeth's Hospital, Washington, DC 20032

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.35	1.35	0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects _(b) Human tissues x_(c) Neither
_(a1) Minors
_(a2) Interviews

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

Neuropeptide FF (NPFF) is the mammalian homolog in the family of peptides related to the molluscan peptide FMRFamide. NPFF has been implicated as an anti-opiate peptide in morphine analgesia, and in the development of tolerance, dependence, and withdrawal. A binding assay for NPFF receptors was developed in membrane preparations of rat brain and spinal cord. The NPFF analog ^{125}I-YLFQPQRFa bound specifically with 0.08 nM affinity to a single population of sites in spinal cord membranes. NPFF receptors were found to be regulated by sodium and magnesium ions and GTP. We found that NPFF receptor binding was highest in brain regions high in NPFF, and the levels of binding in saline-treated and morphine-dependent rats were not different. The NPFF analog daY8Ra reduced morphine tolerance in rats. These results may have implications in treatment of drug addiction and chronic pain.

The proposed course of this project includes (1) study of SAR of peptide binding to NPFF receptors, (2) investigation of the coupling of NPFF receptors to cellular signal transduction systems, (3) development of specific NPFF antagonists, and (4) solubilization and purification of NPFF and FMRFamide receptors.

PHS 6040 (Rev. 5/92)

PROJECT DESCRIPTION

Purpose: The ultimate goal of this work is to determine the role of FMRFamide-related neuropeptides and their receptors in the mammalian CNS. To this end, the immediate objective of this project is to identify and characterize the receptors for FMRFamide-related neuropeptides.

Problem: The tetrapeptide Phe-Met-Arg-Phe-NH_2 (FMRFamide or FMRFa), originally discovered in a marine invertebrate, has led to the discovery of an interphyletic family of related neuropeptides which ranges from molluscs to mammals. The wide distribution of FMRFa-related peptides implies an equally ubiquitous family of related receptors, but information regarding the structure, function, and biochemical coupling of these receptors is very limited. The FMRFa-related neuropeptide in mammalian CNS is an anti-opiate octapeptide of sequence Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2 (FLFQPQRFa, Neuropeptide FF, or NPFF) (Yang et al., 1985, *PNAS* 82: 7757). The mechanism of NPFF action in modulating opioid analgesia, tolerance, dependence, and withdrawal syndrome is unknown.

Scope: Efforts are directed towards characterizing NPFF receptors in mammalian CNS and FMRFa receptors in molluscs. The project includes (1) study of ligand binding to receptors for FMRFa-related neuropeptides, (2) determination of the structure-activity relations of receptor binding to develop specific antagonists, (3) investigation of the coupling of these receptors to cellular signal transduction systems, (4) study of the physiological and behavioral effects of the neuropeptides and their antagonists, including interactions with endogenous and exogenous opioids, and (5) isolation of the receptors from brain tissue.

LigandBinding Studies

We developed an NPFF receptor binding assay in rat CNS using ^{125}I-YLFQPQRFa, a radioligand which has been shown to label a single class of high affinity sites in rat spinal cord membranes (Allard et al., 1989, *Brain Res.* 500: 169). We found this ligand had a much higher ratio of specific to nonspecific binding than an alternative, ^{125}I-daYFLFQPQRFa. We detected NPFF receptor binding in membrane preparations of rat brain as well as spinal cord, and binding was highest in membranes of the dorsal horn. The binding of ^{125}I-YLFQPQRFa to NPFF receptors was saturable (K_d = 0.08 nM), specific, and proportional to the amount of membrane present. We found that optimal specific binding to NPFF receptors in rat brain and spinal cord membranes required 60 mM NaCl and 1 mM $MgCl_2$. The monovalent cation effect was specific in both brain and spinal cord membranes, since neither K^+ nor Li^+ could substitute for Na^+. Neither Ca^{++} nor Mn^{++} could substitute for Mg^{++} in spinal cord, although Mn^{++} was partially effective in brain membranes. The effects of combinations of salts on NPFF receptor binding were highly correlated in brain vs. spinal cord membranes (r^2 = 0.95). Specific ^{125}I-YLFQPQRFa binding to NPFF receptors was examined in membranes prepared from spinal cord and various regions of brain from control and morphine-dependent rats. One group of rats was rendered morphine-dependent by twice daily subcutaneous injections of morphine sulfate (4 mg/Kg) for 7 days. We found the highest specific ^{125}I-YLFQPQRFa binding in dorsal spinal cord, midbrain, medulla oblongata, and hypothalamus. The cortex, cerebellum, and striatum had very low binding. This distribution of receptors correlated with NPFF distribution in these regions (Majane et. al., 1989, *Brain Res.* 494: 1), but no difference

was observed in the levels of receptor binding in morphine dependent vs. saline-treated rats (Akar et. al., *Soc. Neurosci. Abstr.* 18, In Press).

Structure-Activity Studies of Binding

We found that ^{125}I-YLFQPQRFa binding to rat spinal cord membranes was potently displaced by NPFF (K_i = 0.2 nM). As in the interaction of FMRFa with its molluscan receptors, amidated NPFF was nearly 10,000-fold more potent than the free acid. Substitution of Arg-amide for the C-terminal Arg-Phe-amide also severely attenuated binding in brain and spinal cord membranes. Neuropeptide Y and γ_1-MSH, which end in Arg-Tyr-amide and Arg-Phe-amide, were able to compete weakly. Unrelated peptides, such as Substance P, CCK and Met-enkephalin, were completely inactive. Reducing NPFF to the tetrapeptide fragment PQRFa caused a 200-fold loss in potency which could be partially recovered with an N-dansyl. We found that FMRFa was at least 10-fold more potent at NPFF receptors than PQRFa. The specificity of tetrapeptide interaction with NPFF receptors was further probed with a series of FMRFa analogs containing each of 20 amino acid substitutions in positions 1, 2, and 3. In a preliminary experiment each analog was tested at 0.2 μM for ability to compete at NPFF receptors in rat spinal cord membranes. We found that NPFF receptors tolerated every substitution in the Phe^1 position except Met and the acidic Asp and Glu. In the Met^2 position most charged substitutions (Lys, Arg, Asp, Glu) caused large losses in potency, whereas other substitutions were tolerated. Cyclopropyl analogs of Met^2-FMRFa were 30 to 200-fold weaker than FMRFa, although these analogs showed enhanced antiopiate activity (Corriere, et al., *Soc. Neurosci. Abstr.* 18, In Press). All analogs substituted at the Arg^3 position were very weak, with less than 40% inhibition at 0.2 μM.

Receptor-Effector Coupling

Based on the presence of NPFF receptors in rat brain and spinal cord, membranes from these tissues were used to begin elucidating the NPFF second messenger systems. We found that hydrolysis-resistant GTP analogs inhibited NPFF receptor binding in rat brain and spinal cord membranes. The order of potency for this interaction (GTP[γ]S > Gpp[NH]p > GTP > GDP >> GMP, ATP) indicated that NPFF receptors are coupled to G-proteins in these tissues (Payza and Yang, *Soc. Neurosci. Abstr.* 18, In Press). Scatchard analysis of radiolagand saturation data showed that the guanine nucleotide effect was a decrease in the number of NPFF receptors, with no effect on the K_d of the remaining receptor population. To investigate the effects of NPFF on the activity of post-G-protein mechanisms, we required a membrane preparation enriched with NPFF receptors with as little extraneous acivity as possible. Since we found that the dorsal horn and its synaptosomes possessed high NPFF binding, membranes prepared from these sites were examined. The basal and forskolin-stimulated adenylate cyclase activity (AC) of these membranes were unaffected by NPFF or morphine, although Dynorphin 1-17 showed inhibition. Further experiments were planned in which cAMP and other second messengers will be examined for NPFF regulation in sliced and intact spinal cord. To obtain an *in vitro* assay of receptor activation even in the absence of known second messenger, we examined ^{32}P-GTPase activity and GTP[γ]-^{35}S binding in rat spinal

cord membranes. Since we observed no effect of either NPFF or opioids on these parameters, we planned to repeat the experiments with synaptosomal membranes.

Effects on Neural Lobe

We examined whether NPFF in the neural lobe has a local modulatory role on vasopressin or oxytocin secretion. We found that dynorphin A 1-8 (1 μM) inhibits high K^+-induced vasopressin and oxytocin secretion from isolated, perfused nerve endings of the neural lobe. This inhibition was reversed by NPFF (2 or 5 μM) as well as naloxone. The data suggest a local anti-opiate role for NPFF localized in the neural lobe.

Behavioral Effects

Previous studies found that low dosage of daYFLFQPQRa (daY8Ra), a putative NPFF antagonist that we had synthesized, greatly relieved the opiate abstinence syndrome induced by naloxone or NPFF (Malin et al., 1991, *Peptides* 12, 1011). Further testing of daY8Ra showed that the peptide also reduced tolerance to morphine in rats (Lake, et al., *Soc. Neurosci. Abstr.* 18, In Press). Since preliminary results showed that daY8Ra interacted only weakly at NPFF receptors in spinal cord, further evidence is required to support its action as an NPFF receptor antagonist. Substiuted amide analogs of daY8Ra were synthesized and found to have higher affinity for NPFF receptors. Experiments were planned in which further analogs will be synthesized, screened for binding, and tested behaviorally as above.

Isolation of Receptors

As a prelude to purification, we examined whether native NPFF receptors could be solubilized in a form allowing ligand binding. Since we found that NPFF receptors belong to the class of G-protein-coupled receptors, we first tested CHAPS, a detergent which has successfully solubilized other members of this superfamily including FMRFa receptors (Chin and Payza, 1991, *Soc. Neurosci. Abstr.* 17, 1357). However, 100,000xg supernatants of CHAPS-solubilized spinal cord membranes failed to show specific NPFF receptor binding. We planned further experiments with digitonin and CHAPSO as detergents, instead of CHAPS. The use of radioactive, irreversible photoaffinity ligands to tag the receptors prior to purification was also explored. We synthesized an NPFF analog containing photoactive parabenzoyl-L-phenylalanine (pbF) at the N-terminal: pbF-[^{125}I]-YLFQPQRFa. However, addition of this hydrophobic group resulted in high nonspecific binding, and no specific binding could be detected. We planned the addition of more hydrophillic groups to the N-terminal to improve the specific to nonspecific ratio.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

Elucidation of the physiology and pharmacolgy of NPFF is central to understanding morphine tolerance, dependence, and withdrawal. Development of the receptor binding assay and an *in vitro* second messenger assay for NPFF analogs are critical tools in this endeavor, as well as in the design and screening of NPFF antagonists. Our development of an NPFF receptor binding assay is the first in the United States, as is our report of modulation of NPFF receptors by guanine nucleotides and cations. It might be significant that receptor binding of this anti-opiate peptide is enhanced by sodium, which has the opposite, inhibitory effect on opioid recptor binding. Identification of NPFF receptors as members of the family of G-protein coupled receptors should aid in their purification. This information is also required for cloning strategies based on homolgy with other receptors of this class. Particularly exciting are the behavioral studies in which the NPFF analog daY8Ra reversed morphine tolerance in rats. The significance of this is the potential therapeutic use of future daY8Ra-related compounds in managing withdrawal in addicts and tolerance in patients.

PROPOSED COURSE OF STUDY

(1) SAR of binding: The receptor binding assay in rat brain and spinal cord membranes will be used to screen NPFF and FMRFa analogs and putative antagonists for binding potency. The assay in squid optic lobe membranes will be used in a like manner to test putative FMRFa antagonists.

(2) Receptor-effector coupling: We will pursue the cellular signal transduction mechanisms to which NPFF receptors are coupled. Based on our binding data with GTP, the studies will be limited to mechanisms known to be activated by G-proteins. Since NPFF failed to modulate AC or its regulation by opioids in spinal cord membranes, its effect on cAMP accumulation in slices and whole cord will be assessed before concluding absence of effect. We will also compare the NPFF regulation of adenylate cyclase activity and cAMP levels in normal and morphine-dependent rats.
Coupling of NPFF receptors to arachidonic acid release (Piomelli et al., 1987, *Nature* 328: 38) and the phosphatidyl inositol system (Gonzales and Crews, 1984, *J. Neurosci.* 4: 3120) will also be examined. The studies on ^{32}P-GTPase activity and GTP[γ]-^{35}S binding will continue with synaptosomal membranes. Since we have established that squid brain FMRFa receptors are coupled to AC, we will use the assay to screen FMRFa analogs for ability to antagonize the effect of FMRFa.

(3) Behavioral effects and antagonist development: Further putative NPFF antagonists will be designed and synthesized. In collaboration with Dr. Malin, these analogs will be tested for ability to block morphine abstinence syndrome and tolerance in rats. In order to improve the method of delivery of daY8Ra, we will make daY8Ra analogs with modifications intended to increase stability and penetration across the blood-brain barrier. These peptides can also be tested for ability to block the effects of NPFF on opioid analgesia in rats.

(4) Isolation of receptors: The NPFF receptor isolation and purification from bovine brain will begin after suitable binding assays with solubilized receptors are worked out. The FMRFa receptor isolation from squid will continue in collaboration with Dr. Chin. The solubilized receptors will be purified from other membrane proteins by chromatographic methods, and then sequenced.

PUBLICATIONS

Fatatis A, Holtzclaw L, Payza K, Russell JT. Hormone secretion rises then declines during elevated cytosolic free calcium in isolated nerve terminals of the rat neurohypophysis. *Brain Res* In Press.

Lake JR, Malin DH, Hammond MV, Brown SL, Sims JL, Arcangeli KR, Moore GM, Payza K. Analog of Neuropeptide FF attenuates morphine abstinence syndrome. *Peptides* 1991;12: 1011-1014.

Payza K, Russell JT . Activation and inactivation of oxytocin and vasopressin release from isolated nerve endings of the rat neurohypophysis. *J Neurochem* 1991;57: 499-508.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02352-06 CBDB

PERIOD COVERED

October 1, 1991 to September 30,1992

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

Prefrontal Cortical Modulation of Subcortical Dopamine System

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

PI:	B. K. Lipska	Visiting Associate	CBDB, NIMH
Others:			
	D. R. Weinberger	Chief	CBDB, NIMH
	I. T. Phillips	Psychologist	CBDB, NIMH
	F. Karoum	Research Chemist	NPB , NIMH

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Lab of Molecular Neuropsychiatry, Neuropsychiatry Branch

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neuroscience Center at Saint Elizabeths, Washington, D.C.

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	3.0	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.)*

We have studied the effects of ventral hippocampal lesion on mesolimbic dopamine system. Since some evidence exists that structural changes in the temporal limbic cortex in patients with schizophrenia may reflect a neurodevelopmental anomaly, we explored the effects of the lesion induced in neonatal rats on behavioral indices of dopaminergic system later in life. Our results revealed that exciotoxic lesion of the ventral hippocampus remains silent until puberty where upon it produces exaggerated behavioral responses to a novel environment and to d-amphetamine. Moreover, rats with neonatal lesions are hyperresponsive to stress. This effect was not present in rats with similar lesions induced in adulthood. In conclusion, we showed that neurodevelopmental events may play an important role in bringing the effects of the subtle structural dysfunction induced perinatally on dopamine mesolimbic circuitry after puberty. This finding may have important implication for our understanding of mechanisms underlying pathophysiology of schizophrenia.

Z01 MH 02360-06 CBDB

Topographic Analysis of Brain Activity

Project not available at time of printing

Z01 MH 02388-06 CBDB

Regional Cerebral Blood Flow in Neuropsychiatric Patients and in Normal Subjects.

Project not available at time of printing

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECTNUMBER

Z01 MH 02399-07 CBDB

PERIOD COVERED

October 1, 1992 to September 30,1992

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

Postmortem Brain Tissue Examination in Neuropsychiatric Disorders

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

PI:	J. E. Kleinman	Deputy Chief,	CBDB, NIMH
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	M. M. Herman	Neuropathologist,	CBDB, NIMH
	T. M. Hyde	Neurologist,Neuroanatomist	CBDB, NIMH
	Y. L. Hurd	Neuropsychopharmacologist,	CBDB, NIMH
	J. Joyce	Pharmacologist,	Department of Psychiatry; UPMS
	C. N. Karson	Chief,	VA Department of Psychiatry; UAMS

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University of Arkansas Medical School, Department of Psychiatry; and
University of Pennsylvania Medical School, Department of Psychiatry

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Neuropathology

INSTITUTE AND LOCATION

NIMH Neuroscience Center at Saint Elizabeths, Washington, D.C.

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5	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.)*

Postmortem studies in neuropsychiatric disorders test hypotheses with regard to schizophrenia, suicide, and addictions. New findings include the following: (1) Autoradiography of schizophrenics, suicides and controls have been done using a series of noradrenergic and serotoninergic ligands. The most interesting findings involve the 5HT ligands. A previously reported decrease in 5HT uptake sites was confirmed in frontal, cingulate and parietal cortex of schizophrenics without changes in more posterior regions such as hippocampus and temporal cortex. Subcortically, an increase in 5HT uptake binding was seen in the striatum. Suicides had decreased reuptake in the more posterior regions such as entorhinal and temporal cortex. 5HT2 receptors were found to be increased in ventral striatum, posterior cingulate, temporal cortex and hippocampus without changes in frontal or anterior cingulate cortex. 5HTIA were increased in posterior cingulate and hippocampus of schizophrenics and the entorhinal receptors cortex and hippocampus of suicides; (2) A second preliminary group of studies involves the use of Western immunoblot analyses of choline acetyltransferase activities (CHAT) in schizophrenia. CHAT was found to be reduced in schizophrenics in pontine tegmentum with normal levels in frontal, occipital and temporal cortex, thalamus, and cerebellar. Glial fibrillary acidic protein was normal in all 6 brain regions of schizophrenics versus controls; (3) A negative study was published with regard to stains for iron binding in basal ganglia schizophrenia. A second negative study involve the measurement of cells and plagues in the nucleus basalis of Meynert of cognitively impaired schizophrenics versus controls. A third negative study involved a search for cytomegalovirus in the brains of schizophrenics. (4) Studies of basal ganglia of cocaine addicts revealed increases in M-RNA for K-opiate receptor (caudate) and dynorphin (patches in putamen) with decreases of encephalin, u-opiate receptors and mazindol binding (caudate and putamen).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02436-05 CBDB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Combined Sinemet and Neuroleptic Treatment in Schizophrenia

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

PI:	D. G. Daniel	Medical Director	CRSB, NIMH
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	D. R. Weinberger	Chief	CBDB, NIMH

COOPERATING UNITS *(if any)*

Clinical Services Branch

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies; Section on Neuropathology

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.33	.33	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 discontinued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02472-04 CBDB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Pathology of Schizophrenia

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

PI: M. F. Casanova	Neurologist and Neuropathologist	CBDB, NIMH
Others:		
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J. E. Kleinman	Deputy Chief	CBDB, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0		

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☐ (a1) Minors

☐ (a2) Interviews

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

This project has been combined with Project Number Z01 MH 02352-06 CBDB.

Z01 MH 02473-04 CBDB

Study of the Development of Prefrontal Dopaminergic Responses to Stress in the Rat.

Project not available at time of printing

Z01 MH 02476-05 CBDB

Clozapine vs. Haloperidol in Treatment Refractory Schizophrenia.

Project not available at time of printing

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02478-05 CBDB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Effect of Amphetamine on Cerebral Blood Flow in Schizophrenia

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

PI:	D. G. Daniel	Medical Director	CRSB, NIMH
Others:	D. W. Jones	Physicist	CBDB, NIMH
	R. Coppola	Senior Engineer	CBDB, NIMH
	J. Zigun	Senior Staff Fellow	CBDB, NIMH
	J. E. Kleinman	Deputy Chief	CBDB, NIMH
	T. E. Goldberg	Neuropsychologist	CBDB, NIMH
	L. B. Bigelow	Senior Scientist	CBDB, NIMH
	K. F. Berman	Staff Psychiatrist	CBDB, NIMH

COOPERATING UNITS *(if any)*

Clinical Research Services Branch

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Clinical Brain Disorders Branch

SECTION

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INSTITUTE AND LOCATION

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

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☐ (a1) Minors

☐ (a2) Interviews

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

This project has been discontinued.

Z01 MH 02480-04 CBDB

Effects of a Selective D 1 Receptor Agonist, SK&F 38393, in Schizophrenia.

Project not available at time of printing

Z01 MH 02551-03 CBDB

Recognition Memory in Schizophrenic Patients

Project not available at time of printing

Z01 MH 02552-03 CBDB

Pentosan Polysulfate as Prophylaxis for Migraine

Project not available at time of printing

Z01 MH 02553-03 CBDB

Single Dose Amphetamine Effects in Schizophrenia

Project not available at time of printing

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02554-03 CBDB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

Clinical Trial of Fluvoxamine in the Treatment of Chronic Schizophrenia

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

PI:	D. G. Daniel	Medical Director	CRSB, NIMH
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	A. Abi-Dargham	Medical Staff Fellow	CBDB, NIMH
	J. R. Zigun	Senior Staff Fellow	CBDB, NIMH

COOPERATING UNITS *(if any)*

Clinical Research Services Branch

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Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.33	.33	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 discontinued.

Z01 MH 02555-03 CBDB

Pemoline Treatment of Deficit Symptoms in Schizophrenia

Project not available at time of printing

Z01 MH 02556-03 CBDB

Neuropsychological and SPECT Study of Closed Head Injury Patients

Project not available at time of printing

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02573-02 CBDB

PERIOD COVERED
October 1, 1991 to September 30, 1992

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

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

PI:	T. E. Goldberg	Special Expert	CBDB, NIMH
Others:	D. R. Weinberger	Chief	CBDB, NIMH
	K. F. Berman	Staff Psychiatrist	CBDB, NIMH
	J. Gold	Psychologist	CRSB, NIMH
	M. F. Casanova	Medical Officer	CBDB, NIMH
	L. B. Bigelow	Senior Scientist	CBDB, NIMH
	D. G. Daniel	Medical Director	CRSB, NIMH
	C. Hagger	IRTA Fellow	CBDB, NIMH

COOPERATING UNITS *(if any)*
Neuropsychiatry Branch, Clinical Services Branch

LAB/BRANCH
Clinical Brain Disorders Branch

SECTION
Section on Clinical Studies; Clinical Services

INSTITUTE AND LOCATION
NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4.0	3.5	.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.)*

Our work has focused on characterizing various facets of cognitive failure in schizophrenia. We have attempted in particular:

1. To examine memory function. Studies are underway which are examining remote or autobiographical memory and implicit memory in a priming paradigm. Other studies involve assessing working memory using the Parson Petersen paradigm with different interference conditions.

2. To examine specificity. We are assessing cognitive functions in twins discordant for affective disorder, twins discordant for schizophrenia, temporal lobe epilepsy, traumatic brain injury (at NRH), and affective disorder.

3. To examine executive function. A number of tasks that involve delayed response and senile learning have been developed both to ascertain performance profiles and to be used as cognitive activators in PET and SPECT.

4. To examine the effects of neuroleptics. We are comparing patients' performance on neuropsychological tests while they are receiving clozapine, haloperidol, and placebo.

5. To examine course. We are assessing schizophrenic and bipolar patients in cross-sectional designs and schizophrenic patients in a longitudinal design.

6. To examine cognitive enhancing strategies. We are examining the effects of amphetamine on attentional and executive tasks.

7. To examine the relation between movement disorder, cognitive impairment, and psychopathology. We are intensively assessing MZ twins with Tourette's syndrome on neuropsychology, psychopathology, and MRI and patients with Tardive dyskinesia and dystonia.

8. To examine the relation to cognition to psychopathology. The Chapman Scales of anhedonia, perceptual aberration, and magical ideation offer the possibility of ascertaining meaningful relations between brain abnormalities and psychological abnormalities.

Z01 MH 02574-02 CBDB

Chronic Amphetamine Administration in Schizophrenia

Project not available at time of printing

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02575-02 CBDB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Microdialysis: In vivo Measurement of Neurotransmitters in the Rhesus Monkey Brain

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

PI:	R. C. Saunders	Research Psychologist	CBDB, NIMH
Others:	B. S. Kolachana	Staff Fellow	CBDB, NIMH
	Y. L. Hurd	Staff Fellow	CBDB, NIMH
	T. D. Smith	IRTA Postdoctoral Fellow	CBDB, NIMH
	D. R. Weinberger	Chief	CBDB, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Brain Disorders Branch

SECTION
Section on Clinical Studies

INSTITUTE AND LOCATION
NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

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 microdialysis technique has been developed to monitor the dynamic extracellular neurotransmitter levels in the Rhesus monkey brain. This technique enables the characterization of the neurochemical and neuropharmacological correlates, of cortical regulation of subcortical structures, and frontal and temporal lobe functional interaction. A guide cannula system which aids in the accurate and repeatable placement of probes has been shown to be effective in both sedate and awake behaving monkeys. Extracellular dopamine levels were detected in the caudate nucleus and prefrontal cortex of the sedate monkey, however, DA was much lower and more difficult to detect in cortex than in the caudate. DA found in the neostriatum, was sensitive to both K+ evoked stimulation and to neuronal blockade by tetrodotoxin infusions, confirming that the observed DA levels were neuronally released from the synapse rather than released from damaged synaptic vesicles from insertion of the microdialysis probe. DA release was manipulated pharmacologically by infusion of amphetamine and cocaine through the dialysis probe. In both the cortex and the striatum DA levels were dramatically increased. Remarkably, this observed increase could be seen in the cortex even when prior to the amphetamine or cocaine infusion baseline DA levels were below detection. Recently, we have been able to analyze for the presence of amino acids, such as, glutamate, aspartate, and gaba in our dialysate samples. These were more consistantly detected in the cortex and like dopamine increased significantly with amphetamine and cocaine infusion.

We also examined what effects increasing cortical dopamine would have on subcortical targets of the mesocortical dopamine system. Prefrontal cortical infusion of amphetamine or cocaine resulted in significant reduction in striatal DA release. These data demonstrate that in primates cortico-caudate projections regulate the release of striatal DA. We are now in the process of determining whether this reduction is a result of the increase in cortical DA, or from the increase in amino acids, such glutamate. In addition, we are examining whether other cortico-caudate projections, such as those from the parietal or temporal lobe regions have similar regulatory effects.

In order to investigate neurochemical and neuropharmacological correlates of frontal and temporal lobe function we have extended our dialysis procedures to the awake behaving monkey. In the awake monkey we have been able to establish stable baseline levels for acetylcholine, DA and some of its metabolites, and some of the amino acid neurotransmitters. In contrast to that seen in the sedate monkey DA in the caudate was substantially lower and sometimes more difficult to detect. Acetylcholine, however, was easily found in both areas, but, levels appear to be less than that found in the rat. More interestingly our preliminary data suggest that there may be a strong correlation of glutamate levels with performance on a cognitive task. Thus this would be the first demonstration of localized neurotransmitter turnover correlated with cognitive stimulation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02576-02 CBDB

PERIOD COVERED

October 1, 1991 to September 30, 1992

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

PET Studies of Normal Subjects and Neuropsychiatric Patients

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

PI:	K. F. Berman	Staff Psychiatrist	CBDB, NIMH
Others:	J. Gold	Psychologist	CRSB, NIMH
	T. E. Goldberg	Special Expert	CBDB, NIMH
	D. R. Weinberger	Chief	CBDB, NIMH

COOPERATING UNITS *(if any)*

Clinical Services Branch

LAB/BRANCH

Clinical Brain Disorders Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH Neuroscience Center at Saint Elizabeths, Washington, D.C.

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	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.)*

We have measured regional cerebral blood flow (rCBF) with oxygen-15 and positron emission tomography in young normal subjects during performance of a battery of frontal lobe tasks putatively involving working memory as well as during performance of matched sensorimotor control tasks for each frontal lobe task. The results indicate that tasks involving working memory activate a network including dorsolateral prefrontal and parietal cortical areas in humans. Among the brain areas studied to date, this activation is localized to the inferior frontal gyrus in slices falling between six to eight centimeters above the canthomeatal line and parietal cortex eight to 10 centimeters above the canthomeatal line.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 MH 02623-01 CBDB

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Radioligand Discovery and Development

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

PI:	M. B. Sassaman	Chemist	CBDB, NIMH
Others:	K. S. Lee	Chemist	CBDB, NIMH

COOPERATING UNITS *(if any)*

LAB/BRANCH
Clinical Brain Disorders Branch

SECTION
Section on Clinical Studies

INSTITUTE AND LOCATION
NIMH Neurosciences Center at Saint Elizabeths, Washington, D.C.

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	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.)*

This study is designed to discover and develop new radioligands to be used for neuroimaging by single photon emission computerized tomography (SPECT) and positron emission tomography (PET).

We have developed a rapid radioiodination method for labeling Schering-Plough's D-1 antagonist SCH-23390 ("FISCH"), and are presently involved in a collaborative effort with NIDDK to develop ligands for sigma receptors and for dopamine uptake. Several compounds have been synthesized and are currently being evaluated for their potential use as neuroimaging agents. A compound developed in this laboratory for the purpose of imaging dopamine uptake, N-(4-iodobenzyl)imipramine, lacked potential due to the limited solubility of the free base or any of its salts in aqueous media. We are now focused primarily on the synthesis and evaluation of several compounds targeted for the N-methyl-D-aspartate subclass of glutamate receptors.

Current "production line" projects involve radioiodination of IQNB (muscarinic receptor ligand) and IBZM (D-2 ligand) for use in patient studies.