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**The College on Problems  
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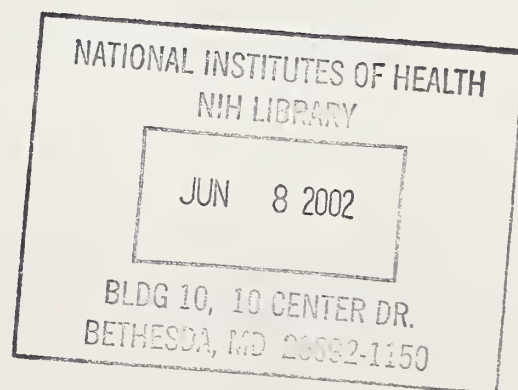


# **Problems of Drug Dependence, 2001:** Proceedings of the 63rd Annual Scientific Meeting, The College on Problems of Drug Dependence, Inc.

## **Editor:**

Louis S. Harris, Ph.D.  
Virginia Commonwealth University

**NIDA Research Monograph 182**



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## INTRODUCTION OF THE NATHAN B. EDDY MEMORIAL AWARD RECIPIENT

*J.H. Woods<sup>1</sup>, J.W. Lewis<sup>2</sup>, and E.L. May<sup>3</sup>*

<sup>1</sup>University of Michigan, Ann Arbor, MI, <sup>2</sup>Bath University, Bath, U.K.  
and <sup>3</sup>Virginia Commonwealth University, Richmond, VA

Kenner C. Rice is the recipient of the Nathan B. Eddy Award in 2001. Dr. Rice has had a distinguished career as a medicinal chemist. He has many outstanding contributions as a researcher, teacher, and strong professional supporter of his discipline. We would like to emphasize each of these aspects of his career.

Kenner's first and very important contribution to narcotics research was a workable, total synthesis of morphine. Chemists had tried for over a half-century to accomplish this task without success. As you know, morphine is most easily derived from the opium poppy, but Kenner's synthesis allowed the possibility that the medical supply of morphine could be made without poppy growth. In addition, his scheme allowed for the synthesis of a variety of important isomers of narcotics, which has in turn allowed the investigation of the pharmacology of asymmetry of a large number of ligands at different opioid and non-opioid receptor sites. His method continues to be used, remains state-of-the-art, and a firm is utilizing it in the process of the development of opioid products. This work alone makes him a chemist recognized internationally as truly outstanding.

Lately, Kenner has directed his efforts toward opioid ligands that can be used for neuroimaging. He has developed some of the most interesting and widely used opioid agents in the field. In addition, he is currently working toward agents that can be used for corticotropin-releasing-hormone receptor imaging; there are none! He has been instrumental in developing irreversible inhibitors at receptors, since ligand covalency of these inhibitors provides that these receptors can be bound tightly and, in principle, could be depleted. This is a technique in pharmacology that affords comparison to short-term use of antisense oligonucleotides and conditional gene knockouts for receptor depletion and, of course, long-term gene knockouts, as well.

He has conceived new templates for opioid receptor types and has been a chemical leader in the synthesis of highly selective opioids. For example, in delta-opioid-receptor pharmacology, a field where non-peptide ligands are very useful, Kenner has designed and directed the synthesis of a large number of ligands, including SNC80, a highly-selective, non-peptide, delta-opioid-receptor agonist that has become a prototype in the field.

He works extensively in other areas of CNS function in addition to opioids. Kenner has made important contributions to the PCP-NMDA-type glutamate receptor field, to novel anticonvulsants, potential pharmacotherapies for cocaine, and ligands for the cannabinoid-receptor system.

Kenner heads the laboratory that was established by Nathan B. Eddy at NIH. It contains a large and very active set of medicinal chemists. He teaches by supervising the research of postdoctoral trainees, and probably very importantly, by having the superb bench skills that others can access directly who work with him. He has high standards of professionalism and ethics, and he imparts these standards well to his trainees. He has had over 50 postdoctoral fellows from 14 countries during his NIH tenure, many of whom have become successful in academia, government, or pharmaceutical industry. To name but a few with whom we're familiar, Amy Newman (NIDA), Mike Rafferty (Warner-Lambert), Brian DeCosta (U. Toronto), Andy Thurkauf (Neurogen), Jim Monn (Lilly), Sylvia Calderon (FDA), and Andy Coop (U. Maryland).

He has a strong interest in professional affairs, and he has taken a strong role in the College on Problems of Drug Dependence, the American Chemical Society, and the American College of Neuropsychopharmacology. He has served and continues to serve on editorial boards of a number of important journals. He has received a large number of awards in recognition of this outstanding contribution to science, e.g., the award of Medicinal Chemistry Division of American Chemical Society in 1996, and a Research Achievement Award in Medicinal and Natural Products in 1998 from the American Association of Pharmaceutical Scientists.

In the distinguished set of chemists that have received the Nathan B. Eddy Award, Kenner Rice can stand proud as an outstanding contributor to the chemical aspects of neuroscience directly relevant to drug abuse. Indeed, he is probably the most important chemist in the field of the neuroscience of drug abuse.

## NATHAN B. EDDY MEMORIAL AWARD LECTURE

*K. C. Rice*

National Institute of Diabetes, Digestive and Kidney Diseases, Bethesda, MD

### MEDICINAL CHEMISTRY IN THE STUDY OF ADDICTIVE DISEASES

First let me say that it is extremely gratifying to stand here as the 28th recipient of the Nathan B. Eddy Award and also as chief of the research group established by Dr. Eddy more than 60 years ago at NIH. At the same time, I am almost overwhelmed with humility and gratitude to those who helped me achieve our goals during the last 27 years. This is without a doubt the high point of my career and I am extremely grateful to have been selected.

I would like to thank my nominator, Dr. Jim Woods, and also Drs. John Lewis and Everette May for supporting my nomination, the Awards Committee that selected me as recipient and all of CPDD. I am indebted to my long-term NIH colleagues Drs. Arthur Jacobson and Richard Rothman and Ms. Mariena Mattson. I would also like to thank Nancy Lew for her long-term support. Much of the chemical work I will describe was done by about 60 highly talented postdoctoral fellows from 16 countries. It has been a privilege to work with numerous others that have made many important contributions in the biological study of compounds synthesized in our group. Time does not permit me to mention each person and their contributions but to those of you in the audience and those of you that are not, let me say thank you very much. I am greatly appreciative of the long-term support of my program by the National Institute of Diabetes, Digestive and Kidney Diseases and for additional support provided by the National Institute on Drug Abuse. I am indebted to Dr. Carl White and Mallinckrodt, Inc. for long-term and generous contributions of starting materials required for our studies. I am also thankful to have been a student of my Ph.D. advisor, the late Dr. John Dyer. Dr. Dyer, together with Drs. Daniel Dickel and the late Harry Petree of Ciba-Geigy Pharmaceutical Co. (now Novartis), largely trained me in organic chemistry. The training I received under Dr. Dickel's tutelage in the practice of organic chemical synthesis during 1971-1972 has served me well during my career and substantially contributed to the development of the NIH Opiate Total Synthesis.

I am very grateful to two great scientists of drug abuse research, Drs. Nathan Eddy and Dr. Everette May. First to Dr. Eddy who more than any other person was responsible for the foundation on which I began my program. Second, to my mentor in this field, Dr. May, for his confidence in giving me a chance to work in his group and for his guidance, sound advice and friendship for the last 27 years. Without his help, I would have had no chance to be standing here today. I attended my first CPDD Meeting and also the International Narcotics Research Conference in 1975 as a postdoctoral fellow in Dr. May's group at the NIH. At these meetings, I was in awe of Dr. May and the other leaders of the field including Dr. Harris Isbell, the Eddy Award recipient that year. Having been trained only in organic chemistry, this was the first time I began to fully appreciate the power of the combination of organic chemistry and pharmacology.

It never crossed my mind that I might someday become the Eddy Award recipient. What did cross my mind was that organic chemistry could play a powerful part in drug abuse research and that I would try my best. In retrospect, I was very fortunate to have entered the field just one year after biochemical identification and binding assays for the "opiate receptor" (then singular) were published and at the time when the endogenous ligands were first identified. I was in attendance at Arlie House in 1975 when Dr. Hans Kosterlitz described two peptides that had been isolated from brain and were active in opioid receptor assays. The structures of these peptides were subsequently elucidated as methionine and leucine enkephalin and the findings published in *Nature* in December 1975. This work, together with the biochemical identification of the receptor, provided much insight into the mechanism of action of opioids at the time. It also greatly extended the opportunity for investigation from what were largely whole animal and isolated tissue studies with some human studies to the quantitative *in vitro* investigation of the receptors and their function. What were needed at that time were diverse chemical tools for elucidation of the structure and function of the opioid receptor-endorphin system. I was certain that with my prior training in organic chemical synthesis I could make a contribution to the field.

Now, as I stand before you almost 20 years to the day after Dr. May received the 1981 Eddy Award, I will relate some highlights of our program which were greatly influenced by my two years as a postdoctoral fellow with Dr. May. First, I would like to briefly mention 11 research areas that we have been involved with since I began in 1974. I will then discuss, in more detail, our work in four of these areas, (a) unnatural opiates and the opiate total synthesis (b) development of cycloxy as a PET ligand (c) delta opioid receptor selective ligands and (d) stimulant abuse-treatment and prevention.

Our contributions to the chemistry of the opium alkaloids were generally aimed at the advancement of our synthetic goals whether this involved improvement of existing synthetic routes by necessity or the development of novel methodology. We developed an improved N-demethylation of morphine and codeine (Rice 1975, Rice and May 1977) and I described a high-yield, boron tribromide mediated O-demethylation of codeine to morphine (Rice 1977), with both methodologies applicable to most other opiate and opiate-related compounds. High-yielding opiate O-demethylation of codeine to morphine was unavailable at the time and was required for our syntheses of unnatural opiate enantiomers from the small amounts of sinomenine then available (see below). This remains an important general transformation in the synthesis of experimental and clinically useful drugs today. Although the O-demethylation was widely applicable, it did not work on compounds highly sensitive to acid conditions such as the thevinols and thebaine itself. We later introduced L-selectride as a convenient reagent for basic O-demethylation of thebaine to oripavine, a transformation that had been unsuccessfully attempted for 60 years (Coop *et al.* 1996, Coop *et al.* 1998). This reagent also proved successful for the acid-sensitive thevinols (Coop *et al.* 1998). This technology is highly complementary to the boron tribromide O-demethylation of opiates I developed earlier and this combination can thus serve for most O-demethylations of opiate and opiate-related compounds. We also developed a practical, direct oxidation of codeinone to 14-hydroxycodeinone, a novel synthesis of thebaine from codeine and a facile synthesis of thebainone-A and dihydrothebainone from codeine. Finally, with regard to our contributions to the chemistry of the opium alkaloids, we developed the NIH Opiate Total Synthesis that will be discussed in more detail below. This is the only practical methodology for the total synthesis of opium-derived medical narcotics and their antagonists and is now in manufacturing process development. Oxide bridge closure in the N-nor series, high-yield oxidation of northebaine to 14-hydroxynorcodeinone and other transformations were developed in this work that greatly extend the versatility of the total synthesis.

Much of our work has involved the design and synthesis of novel drugs as research tools, imaging agents and potential drugs for numerous biological targets. A recurring theme throughout my work in drug abuse research has been the effect of stereochemistry on drug activity. One of my earliest projects in this area was a 1976 study of the antinociceptive effects of thujone and its derivatives (Rice and Wilson 1976). Thujone is a major active constituent of absinthe, an intoxicating essential oil preparation popular in Europe in the period around 1900 that produced very bizarre effects in certain individuals. This project originated after a paper appeared suggesting that the effects of absinthe resulted from the action of thujone enol on the same receptor (then unknown) as  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), the major active principal in marijuana. I recognized that this could not be the case since the absolute configurations of isothujone and  $\Delta^9$ -THC were known and this hypothesis would require the unnatural enantiomer of thujone enol. Since a commercial mixture of (-)-3-isothujone and (+)-3-thujone did however, show significant antinociceptive activity like  $\Delta^9$ -THC, we prepared pure samples of (-)-3-isothujone, its racemate, its epimer (+)-3-thujone and a number of related compounds and studied these for antinociceptive activity in the same systems that  $\Delta^9$ -THC is active. Our results showed that (-)-3-isothujone was the most active compound studied being about twice as active as the racemate and substantially more active than the other compounds in the series and that it produced convulsions at higher doses. Based on these observations of structural and stereoselectivity in the antinociceptive activity of (-)-3-isothujone, we suggested that a specific receptor interaction could be involved. A recent rigorous study published by others showed that (-)-3-isothujone acts as a functional antagonist of the GABA<sub>A</sub> receptor complex and produces convulsions similar to picrotoxin (Hold *et al.* 2000).

We designed and synthesized a number of tools for the central benzodiazepine receptor complex including the first electrophilic affinity label (Rice *et al.* 1979),  $\beta$ -carbolines as inverse agonists, chiral barbiturate enantiomers, chloride channel affinity labels and fluorescent ligands. We studied peripheral benzodiazepine receptor ligands and prepared affinity label enantiomers and an iodinated ligand as a potential imaging agent for single photon emission computed tomography (SPECT) of the peripheral receptor. Our work in the "diazepam insensitive receptor" system involved the design and synthesis of a [<sup>11</sup>C]-labeled drug as a potential agent for positron emission tomography (PET), a [<sup>123</sup>I]-labeled ligand as a potential SPECT agent, and other analogs.

Our studies also involved the design and synthesis of ligands for study of the mechanism of action of phencyclidine (PCP), a drug that produced unpredictable, sometimes violently aggressive behavior in certain individuals. We designed and synthesized enantiomeric pairs of PCP derivatives and related compounds, the affinity labels metaphit and fourphit, [ $^{11}\text{C}$ ]MK-801 and [ $^{18}\text{F}$ ]TCP as potential PET imaging agents and numerous other analogs for various studies. We showed that metaphit antagonized the actions of PCP in single cerebellar cell preparations (Wang *et al.* 1986).

In the cannabinoid-receptor area, we initiated a study to map the anatomical distribution of cannabinoid receptors using tritiated CP55,940 a non-classical, high-affinity cannabinoid ligand originated at Pfizer by Drs. Ross Johnson and Larry Melvin (Herkenham *et al.* 1990). These studies provided insight into the question of why large doses of  $\Delta^9$ -THC are non-toxic and a foundation for further work that continues today. We also designed and synthesized affinity labels for the cannabinoid receptor based on the aminoalkylindoles developed at Sterling and CP55,244, another non-classical cannabinoid developed in the Pfizer program. We recently reported a novel class of CB1 and CB2 cannabinoid receptor ligands.

Our studies have resulted in many novel compounds in a variety of structural classes that act on sigma recognition sites. Among these, we described the first radiochemical synthesis of [ $^3\text{H}$ ](+)-pentazocine, an important radioligand for the sigma-1 recognition site and sigma-2 selective ligands from the indolophenylmorphans class.

Most recently, we have studied corticotropin releasing hormone (CRH) receptor antagonists. This system mediates adaptation to stress, and plays a role in the pathogenesis of anxiety and major depression and is involved in drug dependence and withdrawal. Our goals in this area included the development of functional PET and SPECT imaging agents for the CRH receptor and the design and synthesis of other ligands for various studies related to this system. Among these, we synthesized more than two kilograms of a compound originated at Pfizer, that we named antalarmin. This work provided material for many studies that have provided substantial insight into the CRH receptor system. We showed that antalarmin attenuated the CRH mediated stress responses in primates in the intruder paradigm, an intense social stressor (Habib *et al.* 2000). Our results indicated that antalarmin blocks the ability of CRH to promote blastocyst implantation and early maternal tolerance of pregnancy (Makrigiannakis *et al.* 2001). We synthesized a high affinity fluorinated ligand for the CRH receptor and tritiated this compound. Other studies provided a subnanomolar affinity fluorinated analog as a potential PET ligand and iodinated ligands as potential SPECT imaging agents for the CRH receptor. As part of this program, we developed a practical synthesis of precursors of [ $^{11}\text{C}$ ]MDL100,907, a 5-HT $_{2A}$  receptor antagonist required for our CRH receptor studies.

One of the major research areas we have been involved with since 1974, is the development of novel research tools for study of the opioid receptor system. When I began in Dr. May's group, my first assignment was to synthesize the racemic and chiral 2,5-dimethyl-2'-hydroxy-9 $\alpha$ - and  $\beta$ -propyl-6,7-benzomorphans and other derivatives, which were needed for structure-activity studies (Rice *et al.* 1975). As is usually the case, some of the most difficult compounds in a series are generally the last to be synthesized and this was no exception. The problem here was that the required starting material, 3-propyl-4-methylpyridine was not available and several hundred grams were needed. I began my career in opioid research by synthesizing this material from ethyl acetoacetate. Later, we designed and synthesized the selective affinity ligands BIT for the mu receptor, FIT, FAO (Rice *et al.* 1983) and superfit for the delta receptor and UPHIT for the kappa receptor. These compounds proved to be valuable tools for further characterization of the opioid receptor-endorphin system. We tritiated FIT and superfit to high specific activity and employed superfit for purification of the delta receptor to homogeneity from NG108-15 cells (Simonds *et al.* 1985). This enabled the study of the delta receptor eight years prior to the availability of the cloned delta receptor. We also developed iodinated derivatives of naltrexone as potential SPECT imaging agents. Most of our effort on the development of imaging agents focused on the development of (-)-cyclofoxy [6- $\beta$ -fluoro-6-desoxynaltrexone]. The [ $^{18}\text{F}$ ]( $-$ )-cyclofoxy proved successful for human PET imaging studies and will be discussed in greater detail below, as will the role of the NIH Opiate Total Synthesis in the development of cyclofoxy. We also synthesized a number of opioid enantiomeric pairs and tritium labeled some of these, including some of our affinity ligands that were discussed earlier. In other work related to the delta opioid system, we synthesized numerous delta opioid ligands related to SNC80 including [ $^3\text{H}$ ]SNC121 (see below). We identified a novel class of delta selective ligands and a novel structural type of narcotic antagonist based on the 5-phenylmorphans discovered by Dr. May (Awaya *et al.* 1987). In order to gain further insight into the conformational requirements for the opioid activity of some

phenylmorphans enantiomers, we introduced the oxide-bridged phenylmorphans as conformational probes (Burke *et al.* 1984).

One other area that we have investigated is the development of drugs for investigation of the mechanism of action of psychomotor stimulants and as potential medications for the treatment and prevention of psychomotor stimulant abuse. We developed methodology for the synthesis of isomeric mono and dinitroimipramines and desmethylimipramines and for tritiation of 2-nitroimipramine. We designed, synthesized and tritiated an azido derivative of GBR12935 as a photoaffinity label for the dopamine transporter protein (DAT) that enabled us to purify the transporter to homogeneity in 1991. We also developed electrophilic and tritiated labels for this site along with numerous other GBR12909 analogs for this site including those nonselective for the biogenic amine sites. Finally, we introduced GBR 12909 as a potential medication for the treatment of psychomotor stimulant abuse and have developed ultra long-acting derivatives of GBR12909 for the same purpose.

## UNNATURAL OPIATES AND OPIATE TOTAL SYNTHESIS

One of the first problems that I worked on in Dr. May's group was the synthesis of unnatural (+)-morphine as a tool to study the newly discovered opiate receptor. Dr. Mario Aceto, of the Medical College of Virginia, suggested this problem to Dr. May. Although several opiate total syntheses had been described, the most notable being that of Gates and Tschudi, none were applicable to the synthesis of multigram quantities of unnatural (+)-opiates. It was decided to utilize naturally occurring sinomenine as starting material since this alkaloid had the carbon-nitrogen skeleton enantiomeric with the natural opium alkaloids and had already been converted to (+)-morphine and derivatives by Goto using the methodology of Gates in the late steps. The problem with repeating Goto's work was the limited amount of sinomenine available and the very low chemical yields in the late steps resulting in only about 3% overall yield of (+)-morphine from sinomenine. We planned to develop superior methodology for these steps using the corresponding enantiomers freely available from opium products as model compounds and then to apply these results to sinomenine. Dr. May was able to obtain sinomenine for the program from Tanabe Pharmaceutical Co. who graciously prepared it for us by extraction of *Sinomenium acutum*. We then worked out novel methodology for the conversion of dihydrocodeinone to codeine (Iijima *et al.* 1977) and a rapid high yielding conversion of codeine to morphine (Rice 1977) thus eliminating the low yielding steps of the Goto sequence. We then extended this program to prepare (+)-thebaine and converted it to (+)-naloxone via (+)-oxymorphone. In this work, we prepared multigram amounts of (+)-morphine (Jacquet *et al.* 1977), about a gram of (+)-naloxone (Iijima 1978), and lesser amounts of other (+)-enantiomers. These compounds showed between  $10^3$  and  $10^4$  less affinity for the opioid receptors than their enantiomers *in vitro* and in bioassays and showed no opioid effects *in vivo*. These (+)-enantiomers thus proved to be valuable research tools for detecting opioid receptor mediated effects in diverse systems and (+)-naloxone proved essential in our initial PET imaging of the primate opioid receptor as discussed below. Sinomenine later became unavailable and we considered the feasibility of development of a practical total synthesis of opiates. The impetus to develop such a process was two-fold. First, the worldwide opium shortage of 1973-1976 that emphasized the desirability of development of methodology for the production of these drugs by total synthesis as an alternate to reliance on a natural product. This was a severe shortage requiring release of about half of the U.S. strategic materials reserve of opium to domestic processors so that demands for medical narcotics could be met. Four factors contributed to the shortage: (a) Turkey ending production in 1972; (b) Russia buying opium on the world market for the first time; (c) Indian crop failures in 1973-1974; and (d) increased medical demand for codeine. The second consideration was our requirement for a diverse group of unnatural opiates as research tools. Their synthesis would require development of novel methodology and our goal that has now been successively accomplished was defined as follows: A practical total synthesis should: (a) provide 100+ g of optically pure, correctly oxygenated morphinan intermediates per batch beginning on a 1 mole scale in the laboratory; (b) be as short and simple as possible; (c) be clearly amenable to commercial production of any quantity of all opium derived medical narcotics and their antagonists at a reasonable price; and (d) offer independence from natural sources. Regarding the quantity of materials in question, the 2001 Drug Enforcement Administration production quota for all Schedule 2 (medically useful) narcotics for sale was 129,000 kg with about 65,600 kg of thebaine as raw material (for conversion). Since morphine, codeine, and thebaine are the only materials isolated for the production of medical narcotics and their antagonists from opium, a practical synthesis of these materials would allow full access to the entire spectrum of natural and unnatural enantiomers. In 1980, I published a short, non-chromatographic, practical synthesis of racemic nordihydrocodeinone and dihydrocodeinone using a modified Grewe approach. This route employed unprotected phenolic intermediates and novel oxide bridge closure in the N-

nor series that provided optional access to either compound in about 30% overall yield from 3-methoxyphenethylamine (Rice 1980). This methodology was then easily adapted to the synthesis of both enantiomers of these compounds thus providing access to chiral morphine, codeine and thebaine using methodology we and others described earlier. Subsequent work in our group has resulted in simplified methodology for the chiral synthesis of either enantiomer of nordihydrocodeinone and dihydrocodeinone. We converted the latter to the following compounds in the indicated yield from 3-methoxyphenethylamine with the number of isolated intermediates (any intermediate filtered and washed) shown in parentheses: dihydrocodeinone 40% (5), codeine 36% (6), morphine 32% (7) and thebaine 35% (5). Since the pharmacologic profile of opiates is largely determined by the substituent on nitrogen, we developed methodology for the nearly quantitative conversion of chiral nordihydrocodeinone to northebaine and for high yield, direct oxidation of the latter to 14-hydroxynorcodeinone (Rice and Newman 1997). Catalytic hydrogenation followed by boron tribromide O-demethylation gave noroxymorphone. This chemistry allows introduction of any desired nitrogen substituent at any stage in the sequence and eliminates the multistep replacement of the N-methyl substituent of morphine, codeine and thebaine with other substituents. It thus greatly extends the versatility of the opiate total synthesis by allowing one intermediate to serve as precursor for various drugs with different N-substituents. Using this combined methodology, we synthesized the (+)-isomers of oxymorphone, naltrexone, naloxone, nalmefene, nalorphine, etorphine, buprenorphine, diprenorphine, and numerous other unnatural opiate enantiomers. Although we previously confirmed the optical purity of the early tetrahydroisoquinoline intermediate in the total synthesis and thus materials made from it, we independently verified the optical purity of (+)-oxymorphone from the total synthesis by showing its biochemical identity with (+)-oxymorphone from sinomenine in radioreceptor binding assays. We also synthesized unnatural (+)-cyclofoxy, a compound that played an important role in our PET studies. These studies resulted in the first images of opioid receptor occupancy in the living primate brain (see below). In summary, the NIH Opiate Total Synthesis: (a) provides the only practical methodology for the chemical synthesis of all opium derived medical narcotics and antagonists; (b) allows total synthesis of morphine, codeine and thebaine in 32-36% overall yield with only 5-7 isolated intermediates from readily available raw materials; (c) offers an unlimited commercial source of opium-derived narcotics and antagonists independent of foreign sources of opium; (d) enables unlimited production of the unnatural (+)-enantiomers of all opium-derived medical narcotics and antagonists as research tools and drugs; and (e) offers opium poppy eradication as a worldwide strategy for the elimination of illicit heroin production. This technology is now in manufacturing process development and is the only practical methodology available for this purpose despite continuing attempts by many chemists to develop such a process over the last 70 years, and major advances in organic chemical synthesis made during those decades.

## THE DEVELOPMENT OF (-)-CYCLOFOXY AS A PET LIGAND

The opioid receptor endorphin system consists of saturable, enantioselective, high affinity mu, delta and kappa opioid receptor types (and at least two subtypes of each) located in anatomically well defined areas of the mammalian CNS and the numerous endogenous opioid peptides (endorphins) which subservise these receptors. This system mediates the euphoric and addictive effects of narcotic drugs and regulates numerous physiologic and behavioral functions in its normal state, whereas its dysfunction likely results in a number of CNS disorders. PET scanning is a unique, noninvasive technique available for real time measurement of metabolic activity or receptor occupancy in living animals and humans and is thus applicable to the study of the function of this system. We designed and synthesized (-)-cyclofoxy as a candidate opioid receptor, PET imaging agent. This compound bound preferentially to mu receptors with some kappa receptor binding with about 1 nM affinity *in vitro*, functioned as a potent narcotic antagonist *in vivo* [about 10 x (-)-naloxone (Narcan)] and, importantly, was not metabolized in the brain. Autoradiographic studies in brain sections of rats using [<sup>3</sup>H](-)-cyclofoxy revealed that the drug localizes *in vivo* in opioid receptor rich brain regions and labels a population of opioid receptors similar to that labeled by the clinically used narcotic antagonist naloxone. The binding could be removed by washing, was reversible and could be displaced and prevented by (-)-naloxone, but not the pharmacologically inert (+)-naloxone prepared by our total synthesis. The unnatural (+)-isomer of cyclofoxy prepared by total synthesis using methodology described above was also inactive in these systems. In addition, the release of endorphins could be measured with [<sup>3</sup>H](-)-cyclofoxy in the hamster brain *in vivo* strongly suggesting that [<sup>18</sup>F](-)-cyclofoxy might be a useful imaging agent for opioid receptor occupancy in primates. That proved to be the case and we reported the first successful images of opioid receptor occupancy in the living primate brain in 1984 (Pert *et al.* 1984). In this study, we used the 3-acetyl derivative as a rapidly metabolized prodrug of [<sup>18</sup>F](-)-cyclofoxy. This study in baboons revealed extensive localization of [<sup>18</sup>F](-)-cyclofoxy in the opioid receptor rich caudate nucleus and thalamus analogous to the labeling

of these brain regions in the rat with [ $^3\text{H}$ ]-(-)-cyclofoxy. Accumulation of [ $^{18}\text{F}$ ]-(-)-cyclofoxy in these primate brain regions was displaceable by (-)-naloxone (or preventable with prior administration of (-)-naloxone) in a dose-related manner in contrast to the receptor inert (+)-naloxone that had no effect. In our later studies, we used [ $^{18}\text{F}$ ]-(-)-cyclofoxy in order to simplify the tissue distribution analysis. Equilibrium binding studies in conscious humans developed by Dr. Richard Carson at NIH revealed similar localization of [ $^{18}\text{F}$ ]-(-)-cyclofoxy in the caudate nucleus and thalamus analogous to the labeling of these brain regions in the rat and baboon. In order to better understand opioid receptor function and detect more subtle differences between normal and abnormal subjects, we developed methodology for quantitation of opioid receptor density and affinity applicable to human studies. This was achieved by  $B_{\text{max}}$  and  $K_d$  quantitation in 16 primate brain regions through *in vivo* Scatchard analysis of (-)-cyclofoxy binding under rigorously demonstrated equilibrium binding conditions with precise measurement of non-specific binding. The latter was accomplished by two independent methods that were in excellent agreement: (a) PET studies with the receptor-inert mirror image isomer [ $^{18}\text{F}$ ](+)-cyclofoxy above and (b) displacement of specifically bound (-)-cyclofoxy with (-)-naloxone and subsequent measurement of the residual receptor-unrelated [ $^{18}\text{F}$ ]-(-)-cyclofoxy binding, a method easily applicable to human studies. Opioid receptor quantitation in appropriate brain regions of humans in normal, drug-altered and pathological conditions should allow the development of clinical correlates of receptor dysfunction with disease states. These results will enable rapid and routine diagnosis of these disorders, and provide the means to identify and monitor the effects of appropriate drug therapy for disorders of the opioid receptor-endorphin system. The development of (-)-cyclofoxy may have enormous potential for further understanding normal and abnormal brain function including narcotic and cocaine addiction, other drug-seeking behavior, and the "opiatergic tone" of the CNS which modulates the mesolimbic dopaminergic pathway thought to control the rewarding effects of drug abuse and other behaviors that certain individuals find reinforcing. The first steps toward these goals have been realized with our recent study of normal and methadone maintained former heroin addicts with Dr. Mary Jeanne Kreek and associates (Kling *et al.* 2000). This study provided critical information on the binding of [ $^{18}\text{F}$ ]-(-)-cyclofoxy in 13 brain regions and how this accumulation differs in the methadone maintained subjects.

## DELTA OPIOID RECEPTOR SELECTIVE LIGANDS

We began our studies to identify delta selective drugs after the publication of the lead structure of BW373U86 in 1992 by Dr. Robert McNutt then at the Burroughs-Wellcome Laboratories. Pharmacological results for BW373U86, a racemate, indicated that this compound was a high affinity delta ligand that produced some of its effects through the mu receptor. Our initial approach was to prepare the enantiomers of BW373U86 and a series of chiral derivatives attempting to exploit the remarkable effect of stereochemistry on biological activity observed in the opiates. One of the best examples of this is the enantiomers of etorphine. The (-)-enantiomer of etorphine, originally reported by Bentley in 1963, is among the most potent analgesics known, about 7000 times more potent than morphine. By contrast, the (+)-enantiomer (prepared by us via our opiate total synthesis) was inactive in all tests studied for opioid activity and non-toxic at 100 mg/kg, i.p. in the mouse. It is also a potent non-narcotic antitussive about three times the potency of (-)-codeine. One of the most interesting compounds that resulted from our initial delta ligand synthesis studies was SNC 80, a highly selective delta agonist that shows 2000-fold selectivity in binding and bioassays for delta vs. mu receptors (Calderon *et al.* 1994). This work also provided a number of related drugs that are highly delta selective, for which we established structure activity relationships at the cloned human mu and delta receptors. These studies revealed SNC 162 as one of the most delta selective ligands known with a selectivity ratio of delta vs. mu of 8770. We also synthesized a fluorinated SNC 80 derivative with about 900 fold delta selectivity as a potential ligand for imaging delta opioid receptor occupancy by PET, and [ $^3\text{H}$ ]SNC 121 a novel, highly selective delta opioid receptor radioligand with 6000-fold delta selectivity. In other work, we showed that SNC 80 was the most efficacious delta receptor agonist at the cloned human delta opioid receptor among the compounds studied. In studies in the rhesus monkey, we showed that SNC 80 was a systemically active, delta selective agonist with a rapid onset of action and relative low toxicity in comparison to other opioids.

Recent studies by others have shown that moderately selective delta opioid antagonists suppress (a) cocaine seeking behavior, (b) heroin self-administration in rhesus monkeys and (c) the development of tolerance and dependence to the mu agonist morphine. The former two observations strongly indicate that highly selective delta receptor antagonists might be valuable medications for the treatment and prevention of human cocaine and narcotic abuse, and perhaps other undesirable reinforcing behaviors. The latter observation suggests that a drug showing a mu



agonist-delta antagonist profile might produce strong analgesia without producing tolerance and dependence, thus allowing continuous treatment of chronic pain without escalating doses and the inevitable side effects that occur. Dr. Peter Schiller has independently presented convincing evidence that compounds with the mu agonist-delta antagonist profile do indeed retain strong antinociceptive effects and show reduced side effects. These and other intriguing observations now require additional novel, exquisitely selective, non-peptide ligands as research tools to address many questions of fundamental importance concerning function of the mu, delta and kappa opioid receptor subtypes.

## STIMULANT ABUSE TREATMENT AND PREVENTION

The abuse of cocaine is widely recognized to be an extremely serious health and social problem of epidemic proportions for which there is no effective treatment. The extent of the problem is evident from the approximate 760 metric tons of illicit cocaine production valued at about \$20 billion at average 1999 U.S. kilogram wholesale prices. While cocaine production and abuse has stabilized at a very high level, illicit production of methamphetamine in the U.S. is growing almost exponentially with about 7200 individual clandestine methamphetamine laboratories (*20 per day*) closed by Federal, State and local authorities in 1999. Cocaine acts principally by inhibiting the ability of the DAT to return synaptic dopamine to storage vesicles thus increasing synaptic dopamine and mesolimbic dopaminergic transmission leading to the reinforcing effects of cocaine. In the case of methamphetamine, this drug is a substrate for the DAT and when transported into the storage vesicles causes diffusion of dopamine into the synapse and subsequent elevation of dopaminergic transmission. We adapted the approach of design and development of slowly dissociating agents that block the actions of cocaine on the DAT with less intrinsic activity than cocaine (Rothman *et al.* 1989). Such drugs may also block the action of methamphetamine by inhibition of its transport into the storage vesicles. We identified GBR12909 as our lead compound that showed the desired characteristics. Using *in vivo* microdialysis in the rat, we found that while systemically administered GBR12909 produces a modest elevation of intrasynaptic dopamine in the nucleus accumbens, it blocks the large elevation of dopamine by cocaine in a dose-related manner. This approach to treatment and prevention of cocaine abuse has been validated with our finding that GBR12909 prevents cocaine self-administration in rhesus monkeys trained to self-administer cocaine with no effect on normal behavior as measured by food maintained responding (Glowa *et al.* 1995). Studies with repeated administration of GBR12909 have shown sustained therapeutic effects on cocaine self-administration. For example, in a 12-day treatment study with GBR12909, cocaine self-administration was eliminated in monkeys with no effect on food intake. No evidence for the development of tolerance was observed. When the treatment drug was discontinued after 12 days, cocaine self-administration returned to the pretreatment level but could again be eliminated by renewed GBR 12909 treatment. Our PET studies in the baboon have subsequently shown that GBR12909 blocks the accumulation of [<sup>11</sup>C]WIN35428 (a cocaine analog metabolically more stable than cocaine) on the DAT in a dose-related manner. In addition, doses of GBR12909 that prevent cocaine self-administration substantially occupy the DAT as shown by the displacement of [<sup>11</sup>C]WIN35428 (Villemagne *et al.* 1999). We designed and synthesized a racemic 3-hydroxy-3-phenylpropyl derivative of GBR12909 with a binding and uptake inhibition profile nearly identical to that of GBR12909. Conversion of this material to its decanoate ester afforded an ultra long acting cocaine treatment agent in the rhesus monkey. *One dose* of this compound prevented cocaine self-administration in rhesus monkeys without effecting food maintained responding for nearly 30 days (Glowa *et al.* 1996). Recently, our *in vivo* microdialysis studies in the rat showed that two weeks after administration of this decanoate derivative, methamphetamine elevation of dopamine in the nucleus accumbens after acute administration is nearly eliminated. These data suggest that this strategy may be useful in the treatment of abuse of methamphetamine, as well as that of cocaine. We also synthesized and studied the enantiomers of the 3-hydroxy derivative and found nearly identical binding and reuptake inhibition. By contrast, when the hydroxy group was moved to the adjacent carbon atom to provide a 2-hydroxy derivative the *S*-enantiomer was substantially more active *in vitro* and *in vivo* than the *R*-enantiomer although both enantiomers eliminated cocaine self-administration in rhesus monkeys without effecting food intake. GBR12909 is now in Phase 1 clinical trials under the auspices of the National Institute on Drug Abuse. This compound and the enantiomers of the 2- and 3-hydroxy compounds provide an array of potential candidates for human treatment. In addition, the hydroxy compounds can be converted to decanoate ester derivatives to provide long acting drugs possibly with some having different and more favorable side effect profiles than those of the racemic 2-hydroxy material.

In conclusion, I would like to again thank all of my collaborators, past and present, my teachers, the College on Problems on Drug Dependence, the National Institute of Diabetes, Digestive and Kidney Diseases, the National Institute on Drug Abuse, Mallinckrodt, Inc., Glaxo, Searle, and, you the audience, for your kind attention.

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## SYMPOSIUM I

### THE NATIONAL DRUG ABUSE TREATMENT CLINICAL TRIALS NETWORK – CHALLENGES AND OPPORTUNITIES

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#### INTRODUCTION

Recent advances in basic and clinical neuroscience, neuroimaging and genetics are providing a rapidly growing body of evidence about the pathophysiology and treatment of addictive disorders, and it is now widely accepted that these are chronic relapsing conditions. New medical and behavioral treatments have been shown to have efficacy, but this work has been carried out primarily in academic treatment research environments, by highly specialized staff, and with patient populations defined by restrictive inclusion and exclusion criteria. Translating these new treatments to the broader community is an important public health challenge that was highlighted in a 1998 Institute of Medicine Report, *Bridging the Gap between Research and Practice*, and which is now the focus of the National Drug Abuse Clinical Trials Network launched by the National Institute on Drug Abuse in late 1999. The goal of this symposium, at the 63<sup>rd</sup> Annual Scientific Meeting of the College on Problems of Drug Dependence, was to provide meeting attendees with an overview of the Clinical Trials Network and its associated challenges and opportunities.

In an effort to address the gap between research and practice, the Institute of Medicine (IOM) formed a Committee on Community-Based Drug Treatment Research in 1998. Sponsored by CSAT and NIDA, the Committee was charged to identify relevant treatment strategies and promising research approaches, ways for community-based organizations (CBOs) to participate in and use research, technology transfer strategies, barriers inhibiting application of research, barriers inhibiting collaborative research, and strategies to overcome those barriers.

The Committee found that:

- research findings can be applied to clinical settings
- most treatment programs do not participate in research
- investigators rarely collaborate with community-based drug abuse treatment
- State and Federal policies often do not consider research findings
- consumers are rarely involved in application of research, and
- research and clinical staff need training.

Moreover, there are four factors that inhibit the diffusion of knowledge:

- the structure of treatment
- client, provider and stakeholder diversity
- stigmatization
- and insufficient information about knowledge transfer in community-based treatment.

The Committee made several recommendations. To link research and practice, the Committee recommended building infrastructure to facilitate research in CBOs and developing research initiatives that include CBOs. To link research, policy and treatment, it recommended that states should support research/practice collaboration and that financial incentives be used to promote adoption of proven treatment strategies. Recommended strategies for knowledge development included determining if consumers receive services that have empirical support and supporting services research. Dissemination and knowledge transfer recommendations included synthesizing research for providers and policy makers and promoting evidence-based treatment guidelines. The Committee recommended informing consumers about effective treatments so that they become better advocates. Training

recommendations included supporting research training in and with CBOs, training health professionals about alcohol and drug abuse and using research findings, and training staff in CBOs to apply research findings.

Consistent with NIDA's millennial goal to improve drug abuse treatment throughout the nation using science as the vehicle, and in response to these IOM recommendations, NIDA issued an RFA in January 1999, to establish the National Drug Abuse Treatment Clinical Trials Network. The mission of the Clinical Trials Network (CTN) is to conduct studies of behavioral, pharmacological, and integrated behavioral and pharmacological treatment in rigorous, multi-site clinical trials to determine effectiveness across a broad range of community-based treatment settings and diversified patient populations and to transfer the research results to physicians, providers, and their patients in order to improve the quality of drug abuse treatment throughout the country.

## **THE NATIONAL DRUG ABUSE TREATMENT CLINICAL TRIALS NETWORK – ORGANIZATION AND STRUCTURE**

The CTN is a national network of Nodes enabling testing of promising treatment strategies in diverse treatment settings and with diverse patient populations. Each Node has a Regional Research and Training Center (RRTC) at its hub and includes several Community-based Treatment Programs (CTPs or CBOs). In the first year, NIDA funded six Nodes (New England, New York, Delaware Valley, Mid-Atlantic, Pacific and Oregon). Eight additional Nodes were funded a year later (Southeastern, Florida, Ohio Valley, Great Lakes, Rocky Mountain, Washington, Long Island, and North Carolina). The CTN is a unique network of researchers and treatment providers, combining providers' input in shaping the research agenda, a cadre of dedicated researchers, a network infrastructure to enhance study enrollment, and a large diverse provider and clinical population.

Beginning in 2000, the CTN began building and maintaining an infrastructure to support its mission and fulfill its mandate. The structure of the CTN bears some similarities to the clinical trials networks targeted on AIDS, cancer, and other areas established by other NIH Institutes. A CTN Steering Committee, which governs the CTN, includes a principal investigator and CTP representative from each Node and two NIDA representatives. This group reviews and approves the research agenda, formulates and monitors policies and procedures guiding the research activities, and oversees communications within the CTN, as well as with the greater scientific community and the public. The Steering Committee oversees the work of a Publications Subcommittee, a Concept/Protocol Review Subcommittee, a Dissemination Subcommittee and a developing number of issue-focused workgroups. A small Operations Committee, which reports to the Steering Committee, oversees day-to-day protocol implementation and management and the activities of a Training Subcommittee, a Data Management Subcommittee, a Quality Assurance Subcommittee, and a Regulatory Affairs Subcommittee, as well as the individual Protocol and Project Teams.

A CTN Ad Hoc Oversight Board, chaired by the NIDA Director, oversees all activities conducted by the CTN. The Board advises NIDA on the programmatic advisability of proceeding with studies proposed by the Network Steering Committee and assists the Institute in prioritizing and approving research concepts. The Data and Safety Monitoring Board (DSMB) oversees and monitors the conduct of the clinical trials to ensure the safety of participants and the validity and integrity of the data. The DSMB, which includes experts from several disciplines, also makes an independent assessment of treatment effectiveness and whether or not a trial will continue. An independent NIDA Protocol Review Board reviews the final draft of all protocols submitted by the CTN Steering Committee for scientific and regulatory approval.

## **THE CTN RESEARCH AGENDA**

Research protocol concepts are generated from CTN Nodes in collaboration with the CTPs, with the goal of creating a study that is relevant to CTP daily practice, likely to be applied after the study ends if results are positive, and pose questions that can be tested empirically. Concepts are initially reviewed by the Concept/Protocol Review Subcommittee, then by the Steering Committee and finally by the CTN Ad Hoc Oversight Board. Criteria for selection include the efficacy/scientific credibility of the proposed research, feasibility to implement in CTPs and via the CTN mechanism, public health significance and special considerations such as relevance to HIV and other infectious diseases or to women and minorities. Once approved, a Lead Investigator is appointed by the Steering Committee and a broadly representative Protocol Team is established. All protocols take advantage of the expertise

of the Regulatory, Training, QA and Data Subcommittees, as well as the PIs at all the Nodes. CTPs are represented on each of these committees and also review protocols in the early stages of development. An iterative protocol development and review process assures broad input and buy-in prior to final review and approval.

While many concepts were considered early on, including adolescents and patients in the criminal justice system, the first wave of CTN protocols, all of which were initiated in 2001, includes three buprenorphine/naloxone protocols, a motivational enhancement therapy protocol, a motivational interviewing (MI) protocol, and two motivational incentives protocols. Altogether, these protocols have been implemented in over 50 CTPs and in all 14 of the Nodes.

A second wave of protocols is expected to be initiated in late 2001 or early 2002, and includes a quantitative and qualitative assessment of CTN programs, a smoking cessation treatment protocol, a buprenorphine/naloxone adolescent protocol, a focused aftercare protocol and a protocol focusing on screening and treatment of infections (HIV, HCV, TB, STDs) amongst participants in substance abuse treatment programs.

A third wave of protocol concepts generated seventeen new proposals in areas ranging from pharmacotherapies to behavioral and family therapies, including proposals emphasizing a number of special populations (e.g., adolescents, patients with AIDS/HIV and HCV, court diverted patients, those with psychiatric and/or medical comorbidity, women and trauma).

Special interest groups that have been set up are beginning to provide a snapshot of the current state of relevant issues, to identify empirically supported interventions, to develop research concepts and appropriate special assessment tools, to develop long range research plans, and to serve as expert resources. Current special interest groups include: HIV/AIDS, Adolescent, Women and Gender, Co-morbidity, Court involved patients, and Pharmacological Treatment.

## **BREAKING DOWN BARRIERS - THE CHALLENGES OF TRANSLATING CLINICAL RESEARCH INTO EFFECTIVE PRACTICE**

Historically, tensions and structural obstacles have inhibited research implementation in CTPs. After extensive input and comment from representatives of the research and CTP communities, it seems clear that the inception of NIDA's CTN initiative is viewed as a potential watershed in bridging the research to practice gap.

Prior to the inception of the CTN, it was common for research to be initiated by and concluded at the university with CTPs' role limited to providing sites and subjects. CTPs often had minimal input and tended to feel subordinate in the relationship. Researchers noted that CTPs were sometimes less than receptive to examination of their habitual practice and therefore shunned research, while CTPs viewed researchers as unacquainted with and disinterested in the realities of community-based practice.

There are many challenges to forging a truly integrated and collaborative process. Functional partnerships follow from mutual acknowledgment of complementary strengths while taking into account differing particular needs and missions. The relevance and sustainability of research must be balanced with the need for research validity and reliability. Additionally, individual program circumstances must be balanced against the need for common language across locations and studies. In addition to "cross-cultural" issues, CTPs also confront significant practical burdens ranging from giving over space and additional "elective" tasks to already overburdened personnel, to staff turnover. The experience of the CTN to date indicates that CTPs will fully participate in the research process when certain conditions are met. The CTP representatives need to be involved in the design from the very beginning, they need to perceive the research as meaningful, relevant and productive, and they need to be assured that the additional costs of research are supported by the grant.

Research dissemination also represents a significant challenge since clinicians have often been more interested in journals describing treatment methods and histories of individuals who have recovered, than in studying the findings of controlled studies such as are published in academic journals. In turn, researchers sometimes dismiss "clinical experience" as meaningless unless it can be supported by a controlled study. Each method can provide valuable information. Clinical observations have led to important and clinically relevant research studies. Some recent

examples are studies showing that integrating psychiatric and medical treatment with substance-focused interventions can improve compliance and outcomes for selected groups of patients, or studies showing strong associations between positive outcomes and participation in self-help groups. The CTN aims to improve treatment by helping researchers better understand the challenges of treatment providers, while also helping providers better understand research communications and methods. The CTN's emphasis on collaboration from the level of protocol development to their implementation and reporting of results will help bridge the gap that was identified in the IOM report.

For example, user-friendly publications such as NIDA Notes will be used to orient CTPs to general principles of research. Developing career track CTP in-house specialists in research could make available regular on-site training for interested staff as well as complement researchers' contributions to CTP education. Similarly, through paid fellowships, researchers could familiarize themselves with the day-to-day issues of treatment. These cross-training experiences would be mutually beneficial and enriching and would constitute another milestone in the collaborative process.

Since CTP staff also value face-to-face contact as a means of promoting understanding and trust, cross-training experiences could be further advanced with this personal ingredient in the education process. Through this trust-building exercise, researchers could also develop a fuller understanding of the norms of clinical cultures and enhanced appreciation of the need for an interactive experience that over time could promote learning and an increased appetite for research.

A key factor in CTP receptivity to research is intrinsic to the organizational culture. Thus, behavior change is more likely to occur in "*learning organizations*" that continually expand their ability to shape their future than organizational cultures that are *entrenched in the status quo* and committed to existing views. However, each new growth experience will increase the potential for openness to change.

The necessary ingredients for the evolution of an enhanced partnership are embodied in the structure of the CTN. Considerable headway has already been realized towards the goal of inclusion through activities ranging from joint selection of research concepts and joint review at all stages of the protocol development process, through to meaningful and efficiently designed studies supported by additional manpower and fiscal resources. CTP/researcher partnerships have been and will continue to be a springboard to effectively integrate new skills into the permanent treatment armamentarium.

## **POLICY AND FISCAL HURDLES TO IMPLEMENTATION OF CLINICAL TRIALS IN THE COMMUNITY**

There are several challenging funding issues facing CTN-affiliated CTPs. CTPs have joined the CTN because of their commitment to advance treatment knowledge and not because of financial benefit. However, CTPs can not be expected to lose money in the conduct of CTN protocols. Thus, contracts between the RRTCs and the CTPs must ensure that basic fairness exists in regard to how research costs and particularly routine care costs are addressed. NIDA and the RRTCs must work to educate third party payers about the profound value of supporting these trials.

A serious risk facing the CTPs is the possibility that in some cases they may not receive full reimbursement for the routine costs of care from third party payers because the protocol may be considered to be investigational or experimental. Not at issue are the research costs of care. Those costs are built into the Cooperative Agreements between NIDA and the RRTCs. It is, however, less clear how much, if any, of the routine care costs are budgeted into the Cooperative Agreements. If third party payers do not reimburse the CTPs for the routine costs of care, it is likely that such costs will have to be paid from the Cooperative Agreements. This will reduce the amount of funding available for the conduct of research and will complicate the relationship between the RRTC and the CTP as they negotiate mechanisms for payment of these costs. In this regard, care must be taken within the Node and across Nodes to ensure that CTPs are treated with consistency in terms of what treatment costs will be allowable to charge against the subcontract between the RRTC and the CTP.

Under NIH policy, the RRTCs and, in turn, the CTPs, are obligated to seek reimbursement for usual patient care costs which is defined as "care that would have been incurred even if the research study did not exist." The policy

states that usual care (routine care) costs will not be supported unless the PI grants an exception based upon a finding that meets the terms of the NIH Grant Policy.<sup>1</sup> It will be important to pay close attention to this during CTN start-up to assure that CTPs across Nodes are treated similarly. The Steering Committee may later need to develop criteria for granting relevant exceptions.

The issue of third party reimbursement of routine care costs emanating from clinical trials has received a good deal of attention over the past several years, largely driven by the changes in financing health care brought about by managed care. The result is that it is now less predictable whether or not a payer will reimburse for routine care under a clinical trial, thus making it more difficult to recruit patients into clinical trials. The moving force behind much of the recent attention to this issue comes from the cancer treatment community. The National Cancer Institute (NCI) has successfully negotiated agreements with DOD, VA and some health plans covering the routine care costs of treatment. NCI's Community Oncology Program, which has some features similar to the NIDA CTN, strictly prohibits the use of grant funds for the clinical care of patients.<sup>2</sup>

NIH has been concerned that the lack of a clear policy by third party payers on the reimbursement of routine clinical care, particularly insurers and Medicare, has had a chilling effect on the recruitment of patients into clinical trials. The NIH has entered into an agreement with the American Association of Health Plans (AAHP) in which the AAHP will encourage its members to reimburse for routine care costs, provided that reimbursement is not substantially higher than what a health plan would incur in providing standard treatment by an in-network provider.<sup>3</sup> As a possible precursor to the problems that CTPs might experience, NIH and the AAHP have not been able to implement the agreement because, among others things, they can not agree on what constitutes routine care costs.

The General Accounting Office has also studied this issue and concluded, to the dismay of NIH, that there is little evidence that recruitment into clinical trials has been adversely affected by the lack of a clearly stated policy on clinical trial reimbursement by insurers.<sup>4</sup>

A more successful outcome has arisen from an IOM study of Medicare policy on clinical trial reimbursement. Here, Medicare limits reimbursement to care that is reasonable and necessary but had not articulated a policy on the reimbursement of care under clinical trials. The IOM found that the Medicare fiscal intermediaries were reimbursing for such costs because, in many cases, they were not aware that treatment was being performed under a clinical trial.<sup>5</sup> Based upon the IOM report, then President Clinton issued an Executive Memorandum authorizing Medicare to pay for routine care costs as well as the costs due to medical complications from such trials. This action may portend similar action by the insurance industry as may be prompted by Congressional interest in the issue.

Since a significant portion of many of the CTPs budgets come from Federal, State and local funding, it is likely that there will be little or no restriction on how they will use those funds to cover the costs of care. The big question will be whether or not the State Medicaid programs will reimburse for such costs, if the State is made aware that the costs were incurred in a clinical trial. The consensus from HCFA Medicaid staff is that Medicaid could represent a problem to the CTPs.

Faced with what could be a substantial funding issue for the CTN, NIDA has embarked on an educational effort to inform managed care organizations and managed behavioral health care organizations about the CTN and the importance of supporting routine care costs. NIDA has entered into an alliance with the American Managed Behavioral Healthcare Association in this educational effort.

The NIH, IOM and GAO reports suggest that the CTPs can take some advance preparatory action with third party payers to help make their case for reimbursement. The CTPs should be prepared to demonstrate the following: that the trial is based upon scientific evidence of efficacy; that the trial is based on a randomized, controlled design, answering "real world questions" of potential importance to payers; the multi-geographical nature of the study; the rigor of protocol selection; the support of other credible organizations like NIH, NIDA and the RRTC; that the treatment was medically necessary and the protocol derived from a rigorous IRB process with informed consent; and the legitimacy of costs claimed as routine care. It will also be helpful as protocols are developed and approved, to clearly identify the categories of routine costs and research costs associated with each protocol.



NIDA and the Steering Committee are well on the way to addressing the issue of funding for routine care costs and through their educational efforts could make an important contribution to funding parity for drug treatment.

## **CHALLENGES AND OPPORTUNITIES**

There are more than enough challenges for the CTN as it attempts to blend research and practice, primarily revolving around expertise, the research agenda and research implementation. With both researchers and practitioners bringing their experience to bear, building mutual trust and respect is critical to the success of the partnership. A balance needs to be found between researchers' need for scientific rigor and practitioners' requirements of relevance and cost (i.e. the likelihood that study findings would be able to be applied). The partnership and the national scope of the CTN creates design challenges as well, in areas such as baseline treatment variations, inclusion/exclusion criteria and the choice of interventions that can be used. Challenges for implementation include making protocols user friendly, taking a bottom line approach, using common language, effective training, using common assessment batteries, and open vs. blinded results to therapists.

These challenges are viewed as opportunities as the CTN has great potential for applied treatment research, including research on treatment delivery, genetics, special populations, medications usage development, behavior therapy transferability, large sample clinical studies, long-term treatment effect studies and more. In addition to treatment research, the CTN platform provides a way to assess cost effective process/strategy payments; practice/organizational adaptation to facilitate transfer of science-based interventions; policies to encourage adaptation; incentives to sustain new practice; and effective training strategies.

The Clinical Trials Network is an ambitious undertaking, requiring a long-term commitment from investigators, CTPs and NIDA. As complex as the partnership is, protocols have been written and put into the field in less than two years. The process has been difficult, but all signs to date are positive that the goals of the CTN can be achieved.

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## SYMPOSIUM II

### GENDER DIFFERENCES IN ANALGESIC EFFECTS OF OPIOIDS

*R. M. Craft and J. P. Zacny, Chairpersons*

Given the widespread therapeutic use and substantial abuse of opioids, understanding the variability in their potency and efficacy that can be predicted by sex of the user will enable us to improve our treatment of individuals who have need of opioids therapeutically, as well as those who use opioids recreationally. The goals of this symposium were: (1) to present a summary of current research on sex differences in opioid analgesia, from "mouse to man" and (2) to discuss critical issues in sex differences research. Because it is still relatively early in the development of this line of inquiry, we know very little about the mechanisms underlying sex differences in opioid analgesia. Therefore, these presentations focused primarily on description of the phenomenon, including variables that influence sex differences in opioid analgesia in the various species studied to date.

#### SEX DIFFERENCES IN OPIOID ANALGESIA IN THE RODENT

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Sex differences in opioid antinociception in rats and mice have now been reported by a number of laboratories, using various assays of acute nociception and various types of opioids. Sex differences in antinociception produced by relatively selective mu opioid agonists appear to be independent of assay type. For example, there are numerous reports of sex differences in morphine-induced antinociception using assays of thermal nociception (hot-plate, tail-Withdrawal, tail-flick), and several using assays of chemical (abdominal constriction) and electrical (shock-jump) nociception, with morphine generally being more potent in males than in females (e.g., Baamonde *et al.* 1989; Kepler *et al.* 1989; Cicero *et al.* 1996; Boyer *et al.* 1998). The same may be true for relatively selective kappa agonists, insofar as there are relatively equal numbers of reports showing sex differences and no sex differences in kappa opioid antinociception, regardless of assay (e.g., Kavaliers & Innes 1987; Kavaliers & Cholieris 1997; Patrick *et al.* 1999; van Haaren *et al.* 2000; Craft & Bernal 2001). At present, studies of sex differences in delta opioid antinociception are too rare to provide a consensus. Rather than assay type, what have emerged as important determinants of sex differences in opioid analgesia in rodents are (1) intensity of the noxious stimulus used in the pain test, (2) genotype (strain) of the subject, (3) efficacy (and perhaps selectivity) of the opioid examined, and (4) estrous stage of females at the time of testing. Sex differences tend to be larger when low to moderate rather than very high intensity noxious stimuli are used, certain strains are examined, low to moderate efficacy opioids are examined, and females are tested in estrus rather than in other stages (Berglund & Simpkins 1988; Kest *et al.* 1999; Cook *et al.* 2000; Mogil *et al.* 2000; Craft & Bernal 2001; Barrett *et al.* in press; Stoffel *et al.* in preparation). All of these factors may interact such that in some cases, no sex differences in opioid potency or efficacy are observed, and in rare cases, opioids are found to be more potent in females than in males.

Sex differences in biological phenomena are often induced by differential gonadal steroid milieu during development (organizational effects) and/or during adulthood (activational effects). Currently, there is some evidence that testosterone and estradiol/progesterone in adult male and female rats, respectively, are responsible for sex differences in opioid antinociception (Banerjee *et al.* 1983; Berglund & Simpkins 1988; Mogil *et al.* 2000; Stoffel *et al.* in preparation). Presumably, gonadal steroids influence one or more aspects of opioid pharmacokinetics and/or pharmacodynamics. For example, morphine may enter the brain in greater amounts in males than in females (e.g., Candido *et al.* 1989; Craft *et al.* 1996), and males and females may differ in metabolism of morphine to active and inactive metabolites (Ratka 1995; South *et al.* 2001). However, the fact that several studies report greater morphine-induced antinociception in males than in females after central administration of drug (e.g., Kepler *et al.* 1989; Boyer *et al.* 1998; Krzanowska & Bodnar 1999; Kest *et al.* 1999) suggests that there may also be sex differences in opioid pharmacodynamics (or central pharmacokinetics). At this point there are few studies demonstrating sex differences in opioid receptor density (see Kepler *et al.* 1991; Candido *et al.* 1992; Mogil *et al.* 1994), but very few investigators have examined this possibility in brain (and none in spinal cord), nor have sex differences in opioid receptor-mediated signal transduction been examined.

In summary, sex differences in opioid antinociception have been observed with a variety of mu, kappa and mixed action agonists, with male rodents generally showing greater sensitivity than females. However, efficacy/selectivity of the agonist, intensity of the noxious stimulus, and genotype and hormonal state of the subject influence the magnitude (and occasionally the direction) of the sex difference observed. Sex differences in opioid antinociception in rodents may be due to activational effects of gonadal steroids, which may influence opioid pharmacokinetics and pharmacodynamics.

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## SEX AND GONADAL STEROID HORMONE LEVELS AS DETERMINANTS OF OPIOID ANTINOCICEPTION IN RHESUS MONKEYS

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Accumulating evidence from studies in rodents suggests that there are sex differences in the antinociceptive effects of opioid agonists. Opioid agonists selective for mu and kappa receptors often have higher potency or produce greater maximal effects in males than in females (Barrett *et al.* 2000; Bartok and Craft 1997; Cicero *et al.* 1996; Cook *et al.* 2000; Craft and Bernal 2001; Kavaliers and Innes 1987). In contrast, mu agonists and mixed-action mu/kappa opioids often produce greater analgesic effects in women than in men (Gear *et al.* 1996; Gear *et al.* 1999; Gear *et al.* 2000). There are numerous procedural differences between these preclinical and clinical studies, and one potentially important variable may be the species of the subjects. For example, rodents and primates have different proportions of mu, kappa and delta receptors, and in particular, primates have a higher proportion of kappa receptors than rodents (Mansour *et al.* 1988). In an effort to bridge the gap between preclinical studies in rodents and clinical studies in women and men, we have examined the role of sex and gonadal hormone levels on opioid antinociception in female and male rhesus monkeys. Three sets of experiments were described. Each experiment used a warm-water tail-withdrawal assay of thermal nociception to assess the antinociceptive effects of opioids.

The first set of experiments (Negus *et al.* submitted) was conducted in gonadally intact male and female monkeys, and the females were studied during the follicular phase of the menstrual cycle, when estrogen and progesterone levels are relatively low. The high efficacy mu agonist fentanyl produced similar effects in females and males, whereas the high efficacy kappa agonist U50,488 was significantly less potent in females. We then hypothesized that one factor that may contribute to sex differences in opioid antinociception is the affinity of opioid ligands for their receptors. This hypothesis can be tested *in vivo* in studies with competitive opioid antagonists, because the relative affinity of a competitive antagonist for different receptor types is proportional to its relative potency as an antagonist of agonists that are selective for those receptor types (Dykstra *et al.* 1988). Our study examined potential sex differences in the relative affinity of the competitive opioid antagonist quadazocine for mu and kappa opioid receptors by comparing the potency of quadazocine as an antagonist of fentanyl and U50,488 in females and males. Quadazocine was equipotent as an antagonist of fentanyl in females and males, but it was significantly less potent as an antagonist of U50,488 in females. These findings are consistent with the conclusion that opioid ligands have similar affinity for mu receptors but lower affinity for at least some kappa receptors in female than in male rhesus monkeys. Our finding that U50,488 was less potent in females agrees with several previous studies in rodents (Barrett *et al.* 2000; Craft and Bernal 2001; Kavaliers and Innes 1987; Van Haaren *et al.* 2000) but contrasts with the finding that mixed action mu/kappa opioids are more effective analgesics in women (Gear *et al.* 1996; 1999; 2000). However, it is interesting to note that both our studies in rhesus monkeys and clinical studies in women and men provide evidence to suggest that there are sex differences in kappa receptor populations.

The second set of experiments (Negus and Mello 1999; Negus and Mello in press) evaluated the effects of gonadal hormones on opioid antinociception in ovariectomized females. The antinociceptive effects of the kappa agonist U50,488, the mu agonist morphine, and the mixed mu/kappa opioids butorphanol and nalbuphine were examined during chronic (4 week) treatment with saline, estradiol (E; 0.002 or 0.01 mg/kg/day), progesterone (P; 0.32 mg/kg/day), estradiol + progesterone (E+P; 0.002 mg/kg/day E + 0.32 mg/kg/day P) or testosterone (T; 0.32 mg/kg/day). Ovarian hormone treatments produced physiological levels of E and P similar to those observed during the luteal phase of the menstrual cycle or during pregnancy, and testosterone treatments produced physiological levels of T similar to those observed in gonadally intact males. Both E and E+P significantly enhanced the effects of U50,488. These results parallel the finding that both pregnancy and hormone-simulated pregnancy (i.e. treatment of ovariectomized rats with a pregnancy profile of estrogen and progesterone) produced a kappa receptor-mediated antinociception in rats and enhanced the effects of U50,488 (Dawson-Basoa and Gintzler 1996). Taken together, these findings suggest that kappa receptor-mediated effects may be especially sensitive to ovarian hormone levels in females and may contribute to changes in nociception that occur during different phases of the menstrual cycle and during pregnancy. In contrast to our results with U50,488, gonadal hormone treatments produced little or no change in the effects of morphine, butorphanol or nalbuphine in ovariectomized females.

The final set of experiments (Negus *et al.* in press) examined opioid effects in gonadally intact male monkeys treated with very high doses of testosterone. Prolonged use of high-dose anabolic-androgenic steroids (AAS) may induce a dependence syndrome, and emerging evidence suggests that AAS effects on endogenous opioid systems may contribute to AAS abuse. Accordingly, we tested the hypothesis that high dose AAS treatment enhances endogenous opioid activity in rhesus monkeys as revealed by 1) tolerance to the antinociceptive effects of the mu agonist morphine and 2) physical dependence as indicated by evidence of opioid withdrawal following administration of the opioid antagonist naloxone. Three rhesus monkeys were treated for 14 days with high dose testosterone (3.2 mg/kg/day), and the effects of morphine and naloxone were examined both before and during treatment. Chronic testosterone administration for 14 days produced a 100-fold increase in mean plasma testosterone levels. However, testosterone treatment did not significantly alter morphine antinociception, and naloxone did not precipitate signs of opioid withdrawal either before or during testosterone treatment. These data do not support the hypothesis that high-dose AAS treatment enhances endogenous opioid activity in rhesus monkeys in a way that produces opioid tolerance or dependence. These results also agree with our finding that testosterone did not alter morphine antinociception in ovariectomized females (Negus and Mello in press).

In summary, our studies suggest that sex and ovarian steroid hormone levels may be important determinants of the antinociceptive effects of the kappa agonist U50,488 in rhesus monkeys. Sex differences in U50,488 antinociception may be due, at least in part, to sex differences in the affinity of opioid ligands for kappa receptors, and our results suggest that opioid ligands may have lower affinity for at least some kappa receptors in females than in males. The effects of U50,488 in ovariectomized females can be enhanced by physiological levels of estradiol alone or in combination with progesterone, suggesting that kappa receptor systems in female rhesus monkeys may be especially sensitive to ovarian steroid hormone levels.

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## **GENDER DIFFERENCES IN OPIOID ANALGESIA IN HUMAN VOLUNTEERS: COLD PRESSOR AND MECHANICAL PAIN**

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**INTRODUCTION.** There are a number of preclinical studies which demonstrate that there are sex differences in the analgesic effects of opioids, including morphine, in both rodents and in primates (e.g., Bartok and Craft 1997; Cicero *et al.* 1997; Negus and Mello 1999), with males being more sensitive to the same dose (equated for body weight) than females. A few clinical studies have examined the issue of sex differences in humans using mixed agonist-antagonists and morphine; in the mixed agonist-antagonist studies, females were more sensitive than males (Gear *et al.* 1996; Miaskowski and Levine 1999), and in the morphine studies, one study found no difference (Gear *et al.* 1995) and another detected greater analgesic effects in females (Sarton *et al.* 2000). In the present laboratory study, we examined analgesic and other pharmacodynamic effects of different mu opioid agonists that are commonly used for postoperative pain, including morphine. We examined three different opioids, examined a range of doses of the opioids, and used two different pain assays because preclinical studies have suggested that these factors (drug, dose, pain assay) are important when examining whether there are sex differences in opioid effects (Bartok and Craft 1997). We examined other pharmacodynamic effects of opioids to determine if sex differences in opioid effects are limited to their analgesic effects, or are a more broad-based phenomenon. The pharmacodynamic measures besides analgesia that were assessed in this study included subjective, psychomotor, and physiological effects.

**METHODS.** In this IRB-approved study, and after giving written informed consent, non-drug-abusing, healthy volunteers (16 males [mean age±SD: 28.1±4.6 yrs] and 15 normally-cycling females [mean age±SD: 26.5±6.5 yrs]) participated in a 4-session study. In each 7.5 h session, subjects received four intravenous injections spaced at hourly intervals. Study drugs were saline; morphine (0, 2.5, 5.0 and 10 mg; total cumulative dose: 17.5 mg/70 kg of body weight); meperidine (0, 17.5, 35 and 70 mg; total cumulative dose: 122.5 mg/70 kg of body weight); and hydromorphone (0, 0.33, 0.65 and 1.3 mg; total cumulative dose: 2.28 mg/70 kg of body weight). Pain was induced by both the cold pressor test and pressure algometry at fixed intervals after each injection (and also every 60 min during a 4-h recovery period). Dependent measures included pain ratings, psychomotor/cognitive and physiological measures, subjective effects, and plasma drug levels.

**RESULTS.** The cold pressor test yielded results indicating there were sex differences in amount of pain relief from the three opioids. A significant Sex effect on change in pain intensity ratings in the three drug conditions relative to placebo (scale from 0-10) was obtained during both the drug administration period ( $p=0.02$ ) and the recovery period ( $p=0.03$ ). During the drug administration period, females on average achieved a 1.4 unit decrease in pain ratings which differed significantly from the 0.6 unit decrease in pain intensity ratings in males. A significant Sex effect was also obtained on change in pain "bothersome" ratings in the three drug conditions relative to placebo (scale from 0-10) during the drug administration period ( $p=0.05$ ), with females reporting greater decreases than males. In contrast, no sex differences emerged in the significant increase in pain threshold and tolerance during opioid

administration in the pressure algometry test. All three opioids induced a number of subjective effects that are prototypic of opioids in non-drug-abusing volunteers, and several sex differences were noted. During the meperidine administration period, adjective checklist ratings of "sweating" were higher in males than in females (Sex X Drug X Time:  $p < 0.05$ ). In fact, 50% of the males but no females reported this effect. During the 4-h recovery period after administration of morphine, females reported higher VAS ratings of "feel bad" and "nauseous", and lower ratings of "like drug effect" than did males (Sex X Drug X Time:  $p < 0.05$ ). During and 24 h after the morphine sessions, significantly more females (67%) than males (38%) vomited ( $p < 0.05$ ). Notably, a number of subjective effects did not differ between females and males, including ratings of "dreamy," "dry mouth," "feel drug effect," "heavy, sluggish feeling," "sleepy," and "skin itchy." Performance on the Digit Symbol Substitution Test was impaired by all three opioids, but degree of impairment did not differ as a function of Sex. Likewise, miosis and respiratory depression were induced by the opioids in a dose-related manner, but were not affected by the sex of the subject. Preliminary analyses of the plasma drug levels indicated comparable hydromorphone and meperidine levels between males and females, and higher levels of morphine in males than in females.

**CONCLUSIONS.** Sex differences in opioid analgesia were assay-dependent, suggesting that with some types of pain, there may be no sex differences in degree of analgesia derived from opioids, and with other types of pain there may be sex differences (*cf.*, Kest *et al.* 2000). As well, the sex differences that were noted in some of the subjective effects of the opioids depended on drug, dose, and time at which the effect was measured. Although there have been studies showing a lower incidence in postoperative nausea and vomiting in males than in females (Sinclair *et al.* 1999; Apfel and Roewer 2000), the present finding that males reported lower nausea ratings and that they were less likely to vomit after morphine than were females is intriguing, and to our knowledge has not been reported in the literature. Similarly, there are no reports in the literature we are aware of which have shown a sex difference in sweating after meperidine administration. Females did not have greater plasma opioid levels, suggesting that the sex differences noted in this study were not due to pharmacokinetic factors (*cf.*, Sarton *et al.* 2000). Finally, the fact that we noticed sex differences in some measures, but not in others, suggests that sex differences in opioid pharmacodynamics are not a global effect. In conclusion, sex differences in opioid-induced analgesia were observed in this study, with females showing a greater effect than males. These findings are in accord with the scanty extant human literature (Gear *et al.* 1996; Miaskowski and Levine 1999; Sarton *et al.* 2000), but are inconsistent with the preclinical literature. The reasons for the discordance between species is not clear, and neither is the mechanism for sex differences in opioid analgesia in general (*cf.*, Cicero *et al.* 1997).

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## SEX DIFFERENCES IN THE ANALGESIC EFFECTS OF KAPPA-PARTIAL AGONISTS/ANTAGONISTS IN THE TREATMENT OF POST-OPERATIVE PAIN

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**INTRODUCTION.** A number of currently available opioid analgesic medications, including pentazocine, butorphanol, and nalbuphine, possess agonist/antagonist or partial agonist actions. These drugs, termed "kappa opioids" in this discussion, are thought to act as agonists at kappa opioid receptors and as competitive antagonists or low-potency agonists at mu opioid receptors (Reisine and Pasternak 1996). In the last several years, our laboratory has investigated sex differences in the analgesic efficacy of kappa opioids in post-operative pain.

**METHODS.** All studies were conducted in young, healthy adults undergoing surgical removal of their third molar teeth by the same oral surgeon. To qualify for the study, all surgeries had to include removal of at least one full or partial bony impacted mandibular molar, a procedure that is associated with moderate to severe post-operative pain. The surgical protocol included administration of the local anesthetic mepivacaine without vasoconstrictor, nitrous oxide, and diazepam. Study drugs were openly administered through an intravenous line in a double-blinded fashion using coded syringes. Patients indicated their pain intensity by marking a 10 cm visual analog scale (VAS) anchored with the words "No Pain" at the left end of the scale and "Worst Pain Imaginable" at the right end. Criteria for administration of the study drug were an elapse of at least 70 minutes after the onset of the local anesthetic and a VAS pain intensity score of at least 2.5 cm. The last VAS score before study drug administration was defined as "baseline" and was subtracted from post-drug administration VAS scores to obtain either negative (analgesia) or positive (anti-analgesia) changes in pain intensity. VAS scores were obtained at 20-minute intervals for three hours after study drug administration.

**RESULTS.** Our initial observation of sex differences in kappa-opioid analgesia was in connection with a study of the GABA<sub>B</sub> agonist baclofen as a potential adjunct for improving both morphine (6 mg) and pentazocine (30 mg) analgesia (Gordon *et al.* 1995). Although baclofen enhanced the analgesic effect of morphine in both sexes, the differences between the groups that received pentazocine were better explained by sex differences in the analgesic efficacy of pentazocine than by enhancement by baclofen. That is, women experienced significantly better analgesia from pentazocine than did men. This finding was confirmed in a further study of pentazocine (Gear *et al.* 1996a). To determine if the sex difference observed with pentazocine analgesia is a general property of kappa opioids, we tested both nalbuphine (10 mg) and butorphanol (2 mg). Women experienced significantly greater analgesia than men with both of these drugs (Gear *et al.* 1996b). A subsequent placebo-controlled dose response study with nalbuphine (5, 10 and 20 mg) revealed that 1) there was no sex difference in response to placebo, 2) the highest dose of nalbuphine (20 mg) did not significantly improve analgesia over lower doses for either women or men, and 3) men who received the lowest dose (5 mg) experienced significantly greater *pain* than did those who received placebo (Gear *et al.* 1999). This last observation suggested the existence of an anti-analgesic effect of nalbuphine, at least in men. In women, the effect of the 5 mg dose of nalbuphine was not significantly different from placebo. Because we had earlier observed that a low dose of the opioid antagonist naloxone (0.4 mg) significantly improved pentazocine analgesia (Levine *et al.* 1988), we administered this dose of naloxone with nalbuphine (5 mg) (Gear *et al.* 2000). While naloxone alone did not produce significant analgesia, the naloxone/nalbuphine combination abolished the sex difference and resulted in dramatically improved analgesia in both women and men compared to the same dose of nalbuphine alone.

**CONCLUSIONS.** Although sex differences in hormonal milieu and/or psychosocial characteristics could potentially explain sex differences in analgesic response to kappa opioids, our data do not support these explanations. Rather, we suggest that in addition to their analgesic effects, kappa opioids activate a naloxone-sensitive anti-analgesia mechanism and that this activation is more efficacious in men than in women. Such a mechanism would explain 1) the sex differences in response to kappa opioids, 2) the enhanced pain compared to placebo experienced by men who received nalbuphine (5 mg), and 3) the ability of naloxone (0.4 mg) to switch the response to nalbuphine (5 mg) from increased pain (men) or lack of analgesia (women) to significant, prolonged analgesia in both sexes. Although this putative anti-analgesia mechanism is activated by kappa opioids, it is not known if it is mediated by kappa opioid receptors; kappa opioids are well-known to possess agonist/antagonist



activity at non-kappa receptors. Likewise, naloxone is well-known to act non-selectively. Despite their long availability, kappa opioids have not been perceived by many clinicians to be as efficacious as other opioid analgesics. Perhaps the existence of this proposed anti-analgesia mechanism has contributed to this perception. Further understanding of ways to prevent kappa opioid-mediated anti-analgesia while allowing the expression of analgesia could significantly enhance the clinical usefulness of these drugs and provide viable analgesic alternatives for patients for whom mu opioids might be contraindicated.

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## SEX DIFFERENCES IN OPIOID ANALGESIA: THE TIP OF THE ICEBERG

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As mentioned in the introduction, this line of research is fairly novel, and thus largely in its descriptive phase. There is consensus at this point regarding the existence of fundamental, biologically-based sex differences in both pain sensitivity and opioid analgesic sensitivity. Still unclear is the generalizability of these sex differences: do they apply to all opioids, against all types of pain, in all stages of the estrus cycle? The granddaddy of the generalizability questions is this: are the sex differences the same in all mammalian species? It is with this question that the field faces its largest challenge at present, since the answer appears to be 'no.' That is, for both mu-opioid and kappa-opioid analgesia, human studies appear to reveal an increased sensitivity of women to these drug classes (Gear *et al.* 1996; Miaskowski and Levine, 1999; Sarton *et al.* 2000; also see Zacny section) whereas the rodent literature (Kest *et al.* 2000; also see Craft section) and now non-human primate literature (see Negus section) reveals the exact opposite pattern.

Although this apparent species difference between humans and even their closest relatives will no doubt complicate translational research, I argue that this field is quite ready to shift its focus from descriptive to mechanism-based studies. Indeed, there are really three separate types of sex differences in opioid analgesia that have been described so far. Whereas the most common demonstration is of potency differences (i.e., males or females displaying more analgesia from a particular dose or doses of drug), differences in efficacy have also been described, in which the asymptotic analgesic effect of a drug is higher in one sex than the other. Clinically, whereas a sex difference in drug potency can be overcome simply by titrating the dose accordingly, a sex difference in drug efficacy may contribute to the decision to switch drug classes altogether. Finally and most importantly, there are now any number of demonstrations of *qualitatively* different neural processing of analgesia in each sex (e.g., Mogil *et al.* 1993; Liu and Gintzler, 2000; Tershner *et al.* 2000). It is this type of sex difference that is both the most amenable to mechanistic

studies and that presents the highest clinical relevance, because differential neural circuitry implies that sex-specific molecular targets may be identified for novel drug development. In addition, it is quite possible that qualitative sex differences in analgesic processing may explain quantitative sex differences in potency/efficacy, since the latter may simply represent the differential output of two separate circuits, rather than a (presumably hormonal) modulation of a circuit common to both sexes.

As a relevant example, kappa-opioid analgesia from U50,488H has been shown to be both sex- and strain-dependent in rats (Barrett *et al.* in press) and mice (Mogil *et al.* 2000). In fact, sex and strain (genotype) interact, such that sex differences in U50,488 analgesia can be seen in some genotypes (e.g., Lewis rats, C57BL/6 mice) but not in others (e.g., F344 rats, SM mice). Kappa-opioid analgesia appears to be mediated by different neurochemical circuitry in male and female rodents, being entirely abolished by *N*-methyl-D-aspartate (NMDA) receptor blockade in males, whereas the phenomenon in females is wholly unaffected by such pretreatment (Kavaliers and Choleris, 1997; Mogil *et al.* 2000). Using an F2 intercross between C57BL/6 and DBA/2 mice, we have identified a sex-specific genetic linkage between U50,488 analgesia and mouse distal chromosome 8 (Mogil *et al.* 2000). A gene in this region plays an important role in the mediation of U50,488 analgesia in female mice, but is irrelevant to U50,488 analgesia in male mice. I believe that the impending identification of this gene will allow mechanistic and hypothesis-based experimentation of sex differences in kappa-opioid analgesia, and opioid analgesia more generally.

Finally, ongoing studies in my laboratory have clearly shown that sex is but one of a host of organismic and environmental factors affecting analgesic magnitude in the mouse. As such, we think that the study of sex differences in analgesia is properly regarded as a subset, albeit an important subset, of the larger issue of individual differences in analgesia. It is the explication of the larger issue that will truly produce revolutionary changes in pain therapy for men and women alike.

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## SYMPOSIUM III

### READY, SET, GO: BRINGING BUPRENORPHINE TO THE U.S. TREATMENT MARKET

*L. Amass and W. Ling, Chairpersons*

The face of opioid addiction treatment in the U.S. will change soon with the Food and Drug Administration approval and introduction of the sublingual buprenorphine-naloxone (BNX) tablet to the U.S. treatment market. The development of buprenorphine and BNX has brought with it groundbreaking U.S. legislation permitting physicians to dispense and prescribe schedule III, IV or V narcotic drugs, or combinations thereof, to patients for opioid maintenance or detoxification treatment. For the first time in U.S. history, patients can be treated with an effective opioid medication in the privacy of the physician's office, i.e., outside the traditional narcotic treatment setting. In this symposium, national and international experts spoke on the developmental history, legislative processes, research experience, training of physicians and community providers, and potential therapeutic impact associated with bringing BNX to the U.S. treatment market.

#### THE DEVELOPMENTAL HISTORY OF BUPRENORPHINE

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Buprenorphine was originally synthesized as an opiate analgesic to replace codeine. Reckitt and Colman had a preparation CODIS (8 mg of codeine and 300 mg of aspirin) that they were looking to replace. An interesting series of compounds, orvinols, had been discovered to be several thousand times more potent than morphine. Drs. John Lewis and Ken Bentley synthesized a series of n-cyclopropylmethyl orvinol analogs as this substitution on the nitrogen had previously been shown to reduce abuse liability. Initial tests in man, by Dr. Arthur Keats, of two compounds of this series, M278 and M285, revealed dysphoria and psychotomimetic effects and were probably kappa agonists.

Dr. Alan Cowan joined Reckitt in 1969. He noted that some of the orvinols had bell shaped dose effect curves in the rat warm water tail withdrawal test and correctly deduced that these compounds would have antagonist properties. Dr. Cowan set up physical dependence tests in rodents and Patas monkeys to differentiate the morphine and cyclazocine withdrawal syndromes. The results yielded two candidates, M6007 and M6029. The former had a cyclazocine like withdrawal while M6029 had neither a morphine nor a cyclazocine withdrawal. M6029 (buprenorphine) was selected for clinical testing. Initial tests of 50, 100 and 200 mcg were performed in the initial Phase I. Other Phase I tests revealed that it had poor oral bioavailability. Thus, the sublingual route was tested and found acceptable.

Buprenorphine was tested in dogs and found to both precipitate and suppress morphine abstinence (Martin *et al.* 1976). Dr. William Martin reconciled the data by concluding that buprenorphine was a partial  $\mu$  agonist.

Dr. Don Jasinski tested buprenorphine in opiate addict volunteers. His studies noted that buprenorphine blocked the effects of morphine, had low physical dependence scores and that naloxone could not precipitate withdrawal. Dr. Jasinski was the first person to suggest buprenorphine would be a good treatment medication for opiate dependence (Jasinski *et al.* 1978).

The first study to demonstrate efficacy of buprenorphine compared 8 mg sublingually to 20 and 60 mg methadone. Buprenorphine reduced opiate use and improved retention relative to 20 mg methadone (Johnson *et al.* 1992).

The first multicenter trial of the buprenorphine efficacy compared 1, 4, 8, and 16 mg doses. The *a priori* comparison was between the 1 mg and 8 mg doses. An orderly dose effect in reducing opiate use was found (Ling *et al.* 1998).

Clinical pharmacology studies of combination of buprenorphine and naloxone were performed in order to determine the ratio of buprenorphine to naloxone for sublingual tablets. Mendelson *et al.* (1999) determined that a 4:1 ratio produced an antagonism of buprenorphine's effects when the combination was administered intravenously. This ratio was selected for the combination tablet.

## PAVING THE WAY: THE DRUG ADDICTION TREATMENT ACT OF 2000

*C. O'Keeffe*

### **Reckitt Benckiser Pharmaceuticals Inc., Richmond, VA**

Asking the Congress to pass a small amendment to the Controlled Substances Act – it seemed so straightforward when we began the effort in the mid 90's. We were working with NIDA to develop buprenorphine for the treatment of opiate dependence. Don Jasinski (Jasinski *et al.* 1978) had first postulated its value and this view was scientifically validated over the next 20 years. Industry is can be slow to listen, and has been especially reluctant to take on addiction research. The reasons for this lack of enthusiasm are primarily economic and secondarily social. Despite scientists' best efforts, NIDA, CSAT, ONDCP, Institute of Medicine studies and a wealth of scientific evidence, there's still a stigma attached to addiction and a public misperception that addiction is bad behavior, not disease.

As many of you are aware, Reckitt & Colman (now Reckitt Benckiser) joined NIDA in this project with a full understanding of the difficulties of developing buprenorphine for this indication. We knew it would be at least a five-year project, and that we would be committing tens of millions of dollars to a product that had not one minute of patent protection remaining. I salute our Board who cooperated in this project because it was the right thing to do.

We knew that to recover a significant portion of our expenditures we would need to get this medication into the mainstream practice of medicine – a goal that certainly seemed achievable in light of the IOM's report of consistent recommendations by the scientific community over 20 years. We also knew that we would need some period of market exclusivity to protect our product once FDA approved it.

It was clear that the methadone treatment paradigm was not the way to get a new treatment to patients or significant numbers of new patients into treatment. While the Narcotic Addict Treatment Act had established a good treatment system, it unfortunately had not been modified to keep pace with the times. Like many laws that are not regularly modernized, it failed to provide for scientific advancements over the decades.

So we faced two challenges. To address the market exclusivity matter, we sought Orphan Drug designation, which was granted quickly in 1994. The second challenge was to amend the Controlled Substances Act to allow physicians to treat patients with buprenorphine in the normal course of addiction medicine practice.

The legislation, however, was not enacted so quickly. In the beginning we considered trying to change the Narcotic Treatment Program regulations through an administrative process, but we were convinced that this would not only be a long process, but that it would be encumbered by all sorts of questions and issues that would interfere with reaching the ultimate goal. The next option was to seek a change in the law. It sounded so simple. Change the law to waive the current requirements for physicians prescribing opiates to treat opiate dependence, leaving the methadone system intact, but expanding treatment possibilities. It was motherhood and apple pie. Or so we thought. Bob Angarola and I, one October afternoon in 1995, wrote the first draft of what we called the Drug Maintenance and Detoxification Act. That first draft simply stated that the requirements of the Narcotic Addict Treatment Act did not apply when a physician treated no more than 20 patients with a Schedule V narcotic.

Looking back on this saga today, I am still amazed. This year, the Congress passed and the President signed into law a huge overhaul of the tax system; the Senate changed leadership; and the House passed a massive education bill, all in the period of a couple of weeks. And it took over five years to enact a tiny amendment to the Controlled Substances Act. It's that juxtaposition of timeframes and events that makes me believe the story is worth repeating. It's a lesson in civics, frustration, and patience (or lack thereof).

We started in 1995 by approaching Capitol Hill offices, principally to explain the issue. In a nutshell, we said, we're going to have this promising new product approved which has the potential to bring a significant number of new patients into treatment, but there will be no market for it, and the medical community won't be able to use it because of current legal requirements. We found several offices, where staff was very receptive to our plight. In the Senate, we found a good friend in Senator Carl Levin, of Michigan, who has a longstanding personal interest in expanding and improving addiction treatment. We also found that Senator Hatch and his staff on the Senate Judiciary Committee, which has jurisdiction over the Controlled Substances Act, to be both interested and approachable. Senator Biden, who had introduced legislation to try to encourage the development of new addiction treatment medications was most interested. The climate in the Senate seemed excellent to promote the notion of modifying the law in a way that would provide physicians and their patients easier access to new treatment products.

On the House side, one of our strong allies was then-Representative Tom Bliley, very well positioned as Chairman of the Commerce Committee, which shares jurisdiction over the Controlled Substances Act with the Judiciary Committee. We were able to persuade several key members of the Judiciary Committee and others on both sides of the aisle, that this was good policy.

The problem was that once the groundwork had been laid, time was running out. It was nearing the end of the congressional year, in 1998, before we had educated enough people and rallied enough support to get something going. And, to make matters worse, 1998 was also an election year and the end of the 106<sup>th</sup> Congress. At that time, it was pretty clear that we would never be able to have the bill enacted using the full legislative route.

It was at this late date that Senate staff suggested an alternate approach, and I bit. They suggested negotiating language with the Administration and then using what's called a "must-do vehicle", that is, a bill not necessarily related to the subject matter, but one that must be signed into law. The current "must-do" bill was a multi-agency appropriations bill. Everyone knew this bill had to pass to avoid shutting down the government. So Senator Hatch's staff, with agreement from the Levin, Biden and Moynihan offices, arranged to have our little provision tucked into this funding bill for Senate action. We spent several hours late one October evening negotiating with HHS, Justice, and the White House over provisions of the bill and reached agreement near midnight. Under House rules, the Chairman of the Committee, with jurisdiction over provisions tucked into an appropriations bill, can object and force removal of the provision. But Chairman Bliley, who supported our provision, did not object. We thought we were home free.

But just as in a marathon, you can reach the home stretch and not make it to the finish line. And that's what happened in 1998. Although Chairman Bliley was willing to let this amendment to the Controlled Substances Act pass as part of the appropriations bill, the senior Democratic member of that Committee, Representative John Dingell was not. He objected to the process, not the policy. The Committee had never held hearings on the matter, and had never formally considered the legislation. This, he said, deprived the members of the Committee of an opportunity to examine the policy, understand it, and either agree or disagree with it. He also noted that appropriations bills are not the place to change health care policy. And so we watched helplessly our life passed before us, the provision was removed from the bill, and the issue died, at least for the time being.

We were daunted, but not immobilized by this setback. We determined to survive and to ultimately prevail, because we knew we had the right policy. But we had learned the lesson that, at least in our case, process matters. And we resolved to follow everyone's rules the next time around.

We began by working again with our friends in the Senate to draft legislation. They also worked with other interested parties, including the Administration – FDA, SAMHSA, NIDA, DEA, and the departments of HHS and Justice weighed in. Others in the field – CPDD, AMTA, AAAP, ASAM, APA, AMA, AOA, and others had concerns and suggestions. You can probably guess by looking at the language of the bill who said what. FDA was concerned that the system could get out of hand unless we placed limits on the number of doctors and patients who initially could participate in the system. DEA worried that they wouldn't be able to get a handle on whether physicians were appropriately registered. SAMHSA was concerned about the impact on their resources and about the potential impact on current methadone clinics.

The bill originally introduced by Senators Hatch, Levin, and Biden provided that physicians qualified to treat opiate-dependent patients would be able to prescribe FDA-approved opiates without being subject to current regulations so long as they certified to their qualifications with the Secretary of HHS, 30 days in advance of treating patients, and treated no more than 20. The bill also provided that the new federal paradigm would not be pre-empted by states for at least a period of 3 years, but gave the Secretary of HHS and the Attorney General ample authority to stop the program if there was significant abuse.

That bill was introduced at the end of January 1999 – not bad, considering our balloon had burst only a couple of months earlier. It was to pass the full Senate in November. Now all we needed was a House bill, agreement between the House and Senate, and we were set.

Well, again, I was a little overly optimistic. It seemed that some of the Democratic staff on the House side were still smarting from the ill-fated appropriations effort of the year before, and we needed to be careful about both process and politics. In the meantime, Congressman Dingell had written a letter to the HHS Secretary, raising a variety of questions and concerns, all of which needed to be addressed before we would find any comfort level in that office. Fortunately, the Secretary's responses were squarely on our side. She argued for changing the paradigm of drug treatment, and de-stigmatizing treatment, and for the promise of new treatment products such as buprenorphine.

We were pleased. But it wasn't until the end of July that the House bill finally introduced. A hearing was held on July 30. Although one witness raised concerns about the impact of a new treatment paradigm on the current methadone system, and another raised the issue of whether insurance would cover new treatments, the witnesses otherwise were quite positive. It was significant that Senators Hatch and Levin crossed the great divide between the Senate and House, and testified in support of the bill. Wes Clarke, testifying for SAMHSA, noted the importance of ensuring that states follow any new federal paradigm from the outset, to make certain it caught hold. He cited the LAAM experience as an example of how not to get new interventions broadly adopted. Another three months passed before the Committee acted and the bill was ready for House consideration.

I was frankly stunned that the legislation didn't make it to the House floor for another 10 months. During that time various changes were made in the bill, including, for example, greater specificity about what makes a provider "qualified." Although State preemption remained a concern for some members, the final language was believed to provide sufficient opportunity after an initial transition period for states to make different rules.

Meanwhile, back in the Senate, there was great interest in a methamphetamine bill that had been introduced by Senator Ashcroft. This interest in shutting down meth labs was shared by many House members as well. This meant both the House and Senate Judiciary Committees wanted to give priority, not to our little "buprenorphine bill" as it came to be known, but instead to focus attention on methamphetamine. Thus, before our hostage bill could be released, some activities on methamphetamine, including hearings in members' home districts had to be undertaken. Furthermore, the members wanted to ensure that the methamphetamine bill would sail through the legislative process. So, as they say in DC, deals needed to be cut regarding the futures for both bills.

Back to the Buprenorphine bill – the day finally arrived, July 18, 2000, that the House considered the buprenorphine bill under "Suspension of the Rules". Under this procedure, only one hour of debate is allowed and no amendments are accepted. It's a little more predictable than a process where multiple amendments can be offered. The risk is that this process does require a vote of 2/3<sup>rd</sup>s rather than a simple majority to pass a bill. This is why the Committee was so concerned that the bill not be controversial. The debate on the bill was short and sweet, and other than a few comments about the need for Congress to act on broader substance abuse legislation, the bill was supported and seemed poised to be passed by the House on a voice vote, when suddenly Chairman Bliley made a motion to require a roll call vote. The vote would occur later in the day; I was already blue from not exhaling, when another glitch appeared. It seemed that the version of the bill printed in the Congressional Record was different from the version that had been considered on the House floor. This administrative error meant the bill would have to lay over until the next day, at least.

The days' layover gave the DEA another crack, and they didn't miss a beat. They immediately contacted the House Judiciary Committee and made a "Hail Mary" attempt to require prescribing physicians to register separately with DEA, or get DEA approval before prescribing. Fortunately, this attempt failed and the bill passed the House 412 – 1.

We were on a roll. The bill was placed on the Senate calendar on July 27. Unfortunately, placing a bill on the calendar didn't mean that I'd be watching CSPAN later that evening and see my little bill pass the Senate. The Senate had other ideas.

On August 5, the Senate Judiciary Committee passed the methamphetamine bill and attached to it the buprenorphine bill. So now the Senate had its own bill, quite different from the House bill, so we were in another muddle. The Senate passed the methamphetamine/buprenorphine bill handily and sent it to the House on January 27, 2000. It was a new millennium and although both House and Senate had passed my little bill, there still was no law.

If you're already incredulous, I'm about to turn you into complete skeptics. While all this was going on, the Hatch, Levin and Biden staff were seeking other vehicles for both the methamphetamine and buprenorphine bills. Ultimately, both bills were included in another so-called "must pass" – a huge bankruptcy reform bill. The House and Senate were in conference on this bill. As you may recall, this bankruptcy reform business was hardly benign, and this conference wasn't a tea party. There is yet one more turn. Senator Levin, who was determined to pass the buprenorphine bill, with or without the methamphetamine bill, was the ranking member of the Senate Armed Services Committee. With the concurrence of Senator Warner, the buprenorphine bill was also placed in the Department of Defense Authorization conference, another "must pass" bill to allow the military to function. So we now had two versions of a stand-alone buprenorphine bill, two versions of a buprenorphine/methamphetamine bill, a buprenorphine/bankruptcy bill and a buprenorphine/guns bill.

Life was looking complex at best; bleak at worst when events took another amazing turn. On May 9, 2000, the House passed a bill, H.R. 4365 to "amend the Public Health Service Act with respect to children's health". Without fuss or fanfare, this combination of several children's health bills was scheduled for action. It was now Chairman Bliley's chance to hop aboard moving train. So, H.R 2634, Mr. Bliley's buprenorphine bill, became part of what came to be known as the "Children's Health Act". The House passed this bill and sent to the Senate. After some essentially behind-the-scenes negotiations, on September 22, the bill was passed by the Senate, with an amendment that was, not surprisingly, the Senate version of the buprenorphine bill with the methamphetamine provisions. That amended bill, of course, had to be sent back over to the House and re-considered. The House passed the bill, exactly as the Senate had passed it, as Public Law 106-310 on September 27, 2000, and on October 17, President Clinton signed it.

For those who still recall some of the earlier details, I note that the bankruptcy bill was still in play here, and so was the Defense Authorization conference, so at the last minute before those bills would have been passed and sent to the White House, the buprenorphine provisions had to be snatched out. By the way, the President vetoed the bankruptcy bill on December 19, 2000 – so we were happy to have been riding another horse.

When this process began, I was 2 inches taller and my hair was much darker. Other than that, we survived the congressional process reasonably intact, and I think we now have, in law, a good process, a good program, and an opportunity to make real changes in addiction treatment to the benefit of hundreds of thousands of patients. I genuinely hope that, as a result of this legislation, other companies will see more opportunity in the development of new addiction treatment pharmaceuticals. I believe we broke a significant barrier, and I'm both impressed and grateful that policy makers in Congress and elsewhere hung in there with us because they, too, believed this was the right thing to do. And as important - I am deeply grateful to the scientists whose contributions and participation in this process helped the Congress and the President come to this decision. Your efforts will benefit untold thousands of patients who won't know who you are. You are truly unsung heroes.

## U.S. MULTISITE EVALUATIONS OF OFFICE-BASED BUPRENORPHINE

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Results from numerous studies have supported the therapeutic utility of buprenorphine as an opiate-dependence treatment agent. Almost all of the efficacy and safety data related to the use of buprenorphine for this indication have been obtained from studies utilizing it in research clinics, opioid substitution treatment programs, clinical

pharmacology laboratories - all environments other than office-based treatment settings. Buprenorphine can be an effective therapeutic alternative to methadone and LAAM, medications which are already FDA-approved for opiate dependence treatment. Buprenorphine may also provide a method for expanding and extending treatment options if it is not constrained to use in traditional opioid-substitution treatment programs.

Most of the previous clinical trials conducted to date assessing the efficacy and safety of buprenorphine have utilized buprenorphine alone in a vehicle of aqueous ethanol, typically 30 or 40%. In the present studies, however, the effects of a BNX combination product administered as a sublingual tablet was evaluated. The optimal combination of buprenorphine and naloxone should, when taken sublingually, preserve the therapeutic effects of buprenorphine, and should be associated with minimal opiate antagonist effects secondary to the naloxone component. When taken parenterally by opiate-dependent individuals, the formulation should precipitate opiate withdrawal to deter further illicit use. Based on studies of different opiate-using populations and assessments of various ratios of buprenorphine to naloxone, the 4:1 ratio has been determined to be optimal.

The use of a BNX combination product in an office-based setting is a very exciting approach to treatment. It is anticipated that BNX will meet a therapeutic need for a first-line treatment for opiate dependence in an office-based milieu. Such a treatment environment may be more acceptable especially to persons seeking first-time treatment and can expand and extend treatment options for these individuals, individuals who have been dissuaded or excluded previously by reasons of choice, treatment availability, or regulations.

## **BRINGING BUPRENORPHINE TO COMMUNITY PROVIDERS: THE NIDA CLINICAL TRIALS NETWORK EXPERIENCE**

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Available medications for opioid addiction treatment in the U.S. include methadone, L- $\alpha$ -acetylmethadol (LAAM), and naltrexone. All are effective and have an important role in treating opioid dependence, but less than 18% of the estimated 810,000 U.S. heroin users access these therapies. Methadone and LAAM are strictly regulated by federal and state laws, severely limiting their availability. Naltrexone does not require special registration, but it lacks opioid-like effects and is of limited interest to most patients. Buprenorphine and BNX are promising alternative therapies for opioid addiction that have been under development in the U.S. for over two decades. Food and Drug Administration approval of BNX, the product planned for use in the U.S. market, is still pending. When approved for treatment of opioid addiction, BNX will be the first agonist substitution therapy in recent U.S. history available for office-based practices and other clinics outside the traditional methadone treatment delivery system.

The NIDA Clinical Trials Network (CTN) is a major, ongoing U.S. initiative to integrate research with clinical practice. In an effort to optimize strategies for detoxifying individuals using BNX in non-research settings while enhancing provider clinical experience with the planned marketed product, three open-label, randomized trials have been initiated across 26 U.S. Community Treatment Programs (CTPs) represented by 13 different university groups. Across the 3 studies, over 1,500 opioid-dependent individuals will participate. Two of the trials evaluate the clinical effectiveness of BNX relative to the non-narcotic treatment standard, clonidine, in inpatient and outpatient settings. These two studies will randomize 720 participants (360 per study). The third study compares a 7, 30 and 60 day taper regimen using BNX following a 1-month stabilization with the product. This study will randomize 1008 participants (72 per site).

The CTPs are represented by a diverse group of settings including drug free clinics, therapeutic communities, University-based programs, health maintenance organizations and narcotic treatment programs. CTP clinical experience with opioid users and narcotic treatment medications varies. Some settings have not had prior experience using narcotic medications and have had limited experience with opioid detoxification while others primarily treat opioid-dependent patients with a full range of approved narcotic and non-narcotic pharmacotherapies. CTP research experience and staffing also varies. While some programs have years of experience participating in research, others have never conducted a medication study; similarly, some programs have been newly staffed with experienced research personnel by associated University partners while others are working with their University counterparts to



train existing clinical personnel to conduct the research. Such overall diversity translates into unique implementation challenges for both researchers and clinicians.

For researchers, challenges primarily revolve around improved understanding of fundamental differences between research and clinical practice. For example, researchers are often insensitive to a program's staffing demands, patient management traditions, and clinic schedules. Challenges abound for clinicians. They must learn the differences between running a clinical versus a research program and to carefully balance the needs and demands of the two. Clinicians must gain understanding of the importance of protocol adherence and recognize protocol violations. Philosophical differences between traditional clinical practice standards and standards mandated by research must be managed. Finally, clinicians must ensure that only trained staff manage research participants, and must display flexibility in clinic scheduling to accommodate protocol demands. Nonetheless, despite the different backgrounds of the researchers and community program providers, this diversity has also been the greatest strength of the CTN endeavor. The CTN thus far has been a tremendously positive learning experience for all involved.

Five of six scheduled sites participating in the inpatient comparison of BNX and clonidine, and all six outpatient sites are open and randomizing participants. To date, implementation has been smooth and no major problems have occurred. The third protocol is scheduled to open in the fall of 2001.

The providers that compose this network of clinics are a major nexus for treatment. The availability of BNX is expected to draw large numbers of previously untreated users into the programs and substantially improve treatment retention. Overall, results of these investigations should yield important recommendations for integrating BNX into clinical practice while also elucidating optimal strategies for detoxifying individuals using BNX.

## **TRAINING PHYSICIANS IN THE USE OF BUPRENORPHINE**

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The limited capacity of the current narcotic treatment system has resulted in roughly 600,000-800,000 untreated opioid-dependent patients in the United States (ONDCP, 1999). The need to expand access to treatment identified by the Institute of Medicine and Federal agencies, has resulted in broad support for the use of physician's offices, including primary care settings, for the treatment of opioid-dependent patients using pharmacotherapies including buprenorphine (ONDCP, 1999; National Consensus Development Panel, 1988; Rettig and Yarmolinsky 1995; O'Connor and Fiellin 2000). As of July 2001, approximately 1200 U.S. physicians have received training in the care of opioid-dependent patients using buprenorphine by three of the organizations designated in the Drug Addiction Treatment Act of 2000 (American Society of Addiction Medicine [ASAM], American Academy of Addiction Psychiatry [AAAP], and the American Osteopathic Academy of Addiction Medicine [AOAAM]).

There is a spectrum of physician experience with treatment of opioid dependence and therefore training physicians in the use of buprenorphine presents a unique set of challenges. Developing clinical competence in the use of buprenorphine requires that trainers take into consideration, among other things, the physician's experience in the treatment of opioid-dependent patients, their understanding of the neurobiologic basis of opioid dependence, the rationale for opioid agonist medication, the role of non-pharmacologic treatments and their sophistication in recognizing and caring for the comorbid medical and psychiatric disorders of opioid-dependent patients. Planning and implementing training designed to foster competence with buprenorphine requires an assessment of the target audience. For instance, an audience with experience providing opioid agonist treatment with methadone or LAAM would require a course primarily designed to teach buprenorphine specific materials. In contrast, an audience of physicians who have never provided pharmacologic treatment for opioid-dependent patients or have provided exclusively brief detoxification or opioid antagonist treatments will require training that is designed to highlight the role of opioid agonist maintenance in selected patients and to foster appropriate patient assessment and selection for an expanded range of treatment options. Finally, physicians whose experience is limited to treating other addictive disorders, caring for medical or psychiatric comorbidities in opioid-dependent patients, or those with no experience with this population will require more extensive and general training regarding a range of topics as outlined in the Buprenorphine Training Curriculum and Practice Guidelines. This latter group of physicians may benefit from additional training experiences and consistent with other realms of medicine, all training in the care of opioid-

dependent patients will benefit from an ongoing mentoring or consultant relationship between a greater and lesser experienced physician (Fiellin *et al.* 2001).

Members of the ASAM, AAAP, and AOAAM have developed two documents with support from the Center for Substance Abuse Treatment (CSAT) that can serve as reference documents for trainers and clinical documents for physicians in the care of opioid-dependent patients with buprenorphine. These are entitled "The use of Buprenorphine in the Pharmacologic Management of Opioid Dependence: A Curriculum for Physicians", and the "Buprenorphine Practice Guideline". These documents cover a variety of topics on the use of buprenorphine in the care of opioid dependence and provide a standard set of slides and material for physician trainings. Topics covered in these documents include; basic and applied opioid pharmacology, buprenorphine pharmacology, buprenorphine effectiveness, buprenorphine treatment protocols, non-pharmacologic treatments for opioid dependence, medical and psychiatric comorbidity in opioid-dependent patients, special treatment populations, patient assessment and clinical management, patient confidentiality, and office management.

The movement of the treatment of opioid-dependent patients into office-based settings has parallels to other aspects of medical practice including the treatment of chronic and relapsing medical conditions such as depression and nicotine dependence. Among the important messages that need to accompany the movement of treatment of opioid dependence to primary care settings are the appropriate role of detoxification strategies, the similarities in relapse rates between addictive disorders and other chronic medical conditions, the efficacy of treatment for depression in primary care settings with newer pharmacotherapies and concomitant targeted quality improvement programs and the rewards of providing opioid agonist maintenance treatment in office-based settings. Recent research on the treatment of opioid dependence in primary care physician's offices with methadone and buprenorphine has highlighted the level of patient and provider satisfaction with this model of care. This level of satisfaction and efficacy will likely provide the impetus for more physicians in a variety of office-based settings to receive training in the use of buprenorphine and other pharmacotherapies for the treatment of opioid-dependent patients.

## **CHANGING THE FACE OF ADDICTION MEDICINE: THE ROLE OF BUPRENORPHINE IN OPIATE PHARMACOTHERAPY**

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When we talk about the face of addiction medicine, we are not only talking about how we, the medical profession, specifically treat our addicted patients but also about how we, as a society, deal with our addicted population. In the U.S., this face changes every couple of decades depending on our national mood. In the present context it seems appropriate to limit remarks to the treatment of opiate dependence as exemplifying how Americans view addicts in general and heroin addicts in particular, and how we treat them. By way of introduction, let us refresh our memory with a glimpse of a face from the past. The Report of the Committee on Narcotic Drug Situation in the U.S. published in JAMA in 1920 typecast addicts as either correctional cases, degenerates, social misfits or otherwise normal and made treatment recommendations based on this typology. This did not come from some radical extremist group; it was the official recommendation of the AMA some 80 years ago.

The modern face of opiate addiction medicine can be dated to the introduction of methadone maintenance treatment in the 1960s. In America, this was a time of relative prosperity and the mood of the nation was one of tolerance. The "flower children" were singing "Lucy in the Sky with Diamonds" and professors at some institutions of higher learning were smoking pot as a matter of fashion. Drug use was on the rise. Speed and heroin were everywhere. Mothers were writing their congressmen wanting to know what the government was doing for their sons who were returning home from Vietnam addicted to heroin. The public mood and political demand were ripe for a solution that would be in tune with societal values and provide relief for the problems at hand.

Methadone, while certainly an effective medication by all scientific measures, did not totally meet societal expectations and it was these expectations that influenced its implementation. The American societal view of addicts then, as now, was that they are basically sick and bad and that they cannot be trusted. In other words, they should be helped, but not too much. Still, the number of heroin addicts on methadone maintenance grew from several thousand

in 1973 to sixty thousand two years later. In the ensuing 25 years, that number barely managed to double. The following three points illustrate how this societal philosophy became interwoven into the delivery of methadone treatment.

First, addicts were to be congregated into "clinics" located in the most undesirable parts of town ("Not In My BackYard"). It was not that there were any data to suggest having addicts congregated in a single place was the best form of treatment. Rather, it was done because no one wanted them running all over the nice neighborhoods and if they were all in one place, it was easier to keep an eye on them. As a corollary, methadone clinics were and are stringently regulated and controlled.

Second, there was great societal fear that methadone would be diverted from the clinics into innocent hands, creating a whole generation of new addicts, so take home doses were strictly limited and controlled. Addicts had to earn and maintain their marks to be allowed take homes. Never mind that this simply encouraged addicts to lie to their counselors since they had to say things were going well to keep their take-homes. Addicts learned to hide from their counselors all the bad things that happened in their lives. This was supposed to cultivate trust and make model citizens out of them. Yet policies have not changed substantially despite 30 years of experience showing that diversion of methadone from clinics has not created any substantial number of primary methadone addicts, nor in any other fashion posed a public health hazard. The reality is that only two kinds of people purchase street methadone: addicts who need it and wouldn't have to resort to street buying if the treatment system were adequate and DEA undercover agents.

Third, it was assumed that it was bad for heroin addicts to take any form of medication, especially a narcotic, and that their only hope was to be off drugs completely. Methadone doses had to be kept low and in many clinics a dose ceiling was imposed. These limitations remain even though indisputable data have shown that an adequate dose of methadone is necessary to achieve clinical effects. Moreover, since it is considered "better" for an addict not to be on any medication the period of maintenance was limited and there were strong advocates for cutting off maintenance after two years. Again, never mind all the data showing that it is disastrous for most methadone patients to go off maintenance. Of course all of this was done in the name of doing good for the addicts. Extremists were even claiming that methadone was a scheme for genocide.

Medication development is not immune from these social forces. The development of LAAM was in large measure a response to the fear of methadone diversion. LAAM was said to be good because "no take homes are needed" which later translated to "no take homes are allowed." Likewise, naltrexone was touted as a perfect medication because it is as close to "no medication" as we can get. We wanted addicts to take naltrexone because they could not feel anything and so it was like not giving them any medication. Nobody bothered to ask the addicts how they would feel about naltrexone until it was way too late in the game. Naltrexone became a "victimless cure."

It is generally agreed that hardcore addicts rarely if ever achieve abstinence by detoxification. But this has not stopped our continued efforts to find more and better ways of detoxifying them since it is consistent with our view that addicts must get off drugs even if they don't want to. It is as though by perfecting the technique of detoxification we can change the nature of addiction. The truth is that detoxification is good for a lot of things, but staying off drugs is not one of them.

The current national mood is mixed. We have just ended a period of relative tolerance during which there was talk of treatment and decriminalization of drug offenses, demand for provision of treatment instead of incarceration, and even allowance for such things as medical use of marijuana. But the new era of politics brings uncertainty. A number of existing laws are being challenged and repealed and how the new regime will go from here is anyone's guess. It is generally felt that the present administration has no great love for the addict. To be sure, again consistent with our ambivalence about addicts, there is the new twist of criminal justice treatment for addiction. Sure enough, addicts are sick. Sure enough, we should treat them. But they have also sinned, so let's keep them in jail.

Now we come to buprenorphine, which against this background seems an acceptable compromise. It has something for everybody. It is said that Jasinski *et al.*'s (1978) initial work on buprenorphine was based on the observation that it had properties simulating both methadone and naltrexone. It is as though we can give addicts something that they like, like methadone, and end up getting them onto something we like, naltrexone. The previous presentations leave

like, like methadone, and end up getting them onto something we like, naltrexone. The previous presentations leave little doubt that from a medical and scientific perspective, buprenorphine is highly effective and safe. Its successful implementation in practice though remains to be seen. I believe the scientific and medical community has come more than halfway to meet societal ambivalence. For example, adding naloxone to buprenorphine in order to reduce its street value and minimize the danger of street diversion. Success in changing the face of addiction medicine must now depend on the enlightenment of those in leadership positions, and on their courage to consider scientific facts and clinical knowledge, as well as the vulnerability and needs of the addicts. We must move forward boldly, for only then can the face of addiction medicine be truly changed.

## DISCUSSANT

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More than 23 years have elapsed since Jasinski and coworkers (1978) first suggested that buprenorphine could be useful in the treatment of opioid addiction, even though generous federal support was granted to develop it for that indication. This long lag time has made it possible to conduct an extensive series of controlled and demonstration studies that should facilitate the smooth introduction of buprenorphine into clinical practice. Charles O'Keeffe has candidly described the ultimately successful effort to persuade Congress to keep buprenorphine separate and free of the burdensome constraints of the federal and state regulations that currently govern the use of methadone and LAAM. Nevertheless, the same social and political forces that have made it extremely difficult to loosen those constraints will undoubtedly continue to influence the acceptance of buprenorphine in a framework of office based treatment. Buprenorphine is not demonstrably superior to methadone or LAAM in reducing the use of heroin, although it is considerably less toxic when consumed in excessive amounts or by non-tolerant persons. Yet, the federal, state, and local regulatory requirements that limit the use of methadone and LAAM to clinics and that have imposed almost unbearable compliance burdens on patients have been virtually eliminated for buprenorphine. Undoubtedly patients will find treatment less demeaning when it is received in a doctor's office rather than at a clinic serving hundreds of patients arriving for their medication at the same time. But, in my view, the major obstacle to the delivery of opioid substitution treatment has not been the burdensome regulations, but the low level of support for this type of treatment. A few states excepted, whenever resources for opioid substitution treatment have been increased, treatment capacity has expanded and more patients have entered treatment. The thorough documentation of the efficacy of this clinic system, with all of its flaws, has provided the rationale for both the development of buprenorphine and the establishment of a legislative basis for a new system of treatment delivery.

When methadone first gained federal support 30 years ago, many people in the Congress and in some agencies of the Executive branch of government were concerned that treating addicts with methadone would supplant providing them with psychological and employment counseling, and that diversion of methadone from patients to the street would lead to new addicts and an increase in overdose deaths. Because of this concern, federal regulations mandated the provision of psychosocial services and strict adherence to procedures aimed at minimizing diversion. The burden on patients was not a paramount concern. For buprenorphine, a new compromise has been struck.

We should have two major concerns about this new compromise. First, physicians who prescribe buprenorphine may not ensure that patients get the psychosocial support that has been amply shown to increase the effectiveness of opioid substitution therapy. As a consequence, in practice, buprenorphine may prove to be less effective in reducing heroin use. Second, it is uncertain who will pay for buprenorphine treatment for those who cannot afford it. If it is not subsidized, some patients may sell part of their medication to finance their own treatment. It is difficult to predict how such behavior will be handled by the media and how the public will react. If such diversion is at all significant, or if the problem is exaggerated, given the ambivalent American attitude toward the treatment of heroin addicts, we are less likely to see an expansion of financial support for buprenorphine treatment than a demand for it to be more tightly regulated.

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## SYMPOSIUM IV

### CELLULAR EFFECTS OF CANNABINOIDS

*W.L. Dewey, Chairperson*

#### ENDOCANNABINOIDS: A NEW CLASS OF SIGNALING MOLECULES IN THE BRAIN

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It has been appreciated for thousands of years that ingestion of cannabis sativa produces physiological and psychological effects. In 1963, Mechoulam and coworkers identified the active principal of marijuana,  $\Delta^9$ -tetrahydrocannabinol (3). The mechanism by which THC affected the brain was not clear until work by Howlett and coworkers provided biochemical evidence that the actions of THC and other cannabinoids were mediated by a G-protein coupled receptor (GPCR) (7). The receptor was cloned in 1990 by Matsuda, Bonner and colleagues and indeed has the molecular characteristics of a GPCR (11).

The discovery of a cannabinoid receptor led to the question of the identity of its endogenous ligand. In 1992, Mechoulam, Devane and co-workers provided evidence that *N*-arachidonyl ethanolamine (trivial name anandamide) is made by brain and binds to the CB1 cannabinoid receptor (1). Subsequent studies support this original observation; anandamide binds the CB1 receptor with moderate affinity and has about 60% the maximal effect (efficacy) of the synthetic cannabinoid Win 55212-2 (9). Anandamide produces cannabinoid-like effects *in vivo*, including hypothermia at intravenous doses between 0.1 and 1.0 mg/kg (6). In this dose range, the hypothermic effects are blocked by the CB1 antagonist SR141716.

In 1995, Mechoulam (12) and Sugiura (13) simultaneously published that 2-arachidonylglycerol (2-AG) also has the characteristics of an endocannabinoid. 2-AG binds to both the CB1 and CB2 receptors with low affinity in membrane preparations; however, these data are likely confounded by catabolism of 2-AG during the binding assay. The estimates of 2-AG affinity in whole cell preparations suggest that it binds to the receptor in the nanomolar range. Unlike anandamide, 2-AG is a fully efficacious agonist of the CB1 receptor (4).

Data from many laboratories support the hypothesis that one or both of these molecules is a true neurotransmitter in the brain, made for the purpose of binding and activating the CB1 cannabinoid receptor. The traditional criteria to be met to accept a molecule as a neurotransmitter include: co-localization of the neurotransmitter with target receptors; a mechanism for the synthesis of the neurotransmitter by cells; evidence that the neurotransmitter is released in response to some kind of stimulation; a mechanism for inactivation of the neurotransmitter and pharmacologic mimicry, meaning that the addition of the neurotransmitter exogenously mimics the effects of endogenous release. A few of these criteria have been satisfied; for example, the mechanisms involved in the cellular synthesis of both anandamide and 2-AG have been described (4). Like other arachidonate-derived signaling molecules, neither anandamide nor 2-AG are stored in vesicles. Synthesis of both molecules is induced by increased intracellular calcium, however, the role of this process in the physiological synthesis of the endocannabinoids is unknown. Recent circumstantial evidence suggests that activation of certain GPCRs, including the calcium sensing receptor and metabotropic glutamate and GABA receptors increase anandamide and 2-AG synthesis through a phospholipase C-dependent process.

Both 2-AG and anandamide are inactivated in cells in culture by a combination of reuptake via a facilitated diffusion, carrier mediated process and catabolism by fatty acid amide hydroxylase (FAAH), and in the case of 2-AG, other lipases in the cell. The anandamide carrier protein has not yet been identified molecularly but has been well-characterized biochemically (5). Several inhibitors of the carrier have been identified, including AM404 that have proven useful *in vitro* and *in vivo* (8). FAAH has been well studied as well and its distribution in brain is very interesting. In many brain regions, FAAH is found in cells that are across the synapse from cells expressing the CB1 receptor suggesting that it may label endocannabinoid synthesizing cells in a manner analogous to monoamine-

labeling of catecholamine synthesizing cells (14). FAAH has several substrates, including the sleep-inducing lipid oleamide.

The advent of highly sensitive methods for the measurement of the endocannabinoids has allowed for studies of changes in endocannabinoid concentrations in brain with various treatments. For example, leptin treatment results in an increase in hypothalamic anandamide and 2-AG levels (2). Formalin-induced pain responses are accompanied by an increase in anandamide recovered in the periaqueductal gray via a microdialysis probe (15). Indirect evidence for the release of endocannabinoids during rapid neuronal firing has been obtained by several laboratories, including Kreitzer and Regehr who demonstrated that the CB1 receptor antagonist AM 251 inhibited the production of retrograde inhibition at glutamatergic synapses on cerebellar Purkinje cells (10). This and other studies suggest that one role for the endocannabinoids in the brain is in the negative regulation of synaptic activity via effects on neurotransmitter release.

In conclusion, the endocannabinoids are very interesting molecules. The analytical techniques and other important tools for their study have been developed over the last few years that will allow for further growth in our understanding of the role of the endocannabinoids and the CB receptors in the regulation of neuronal activity in the brain.

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## EFFECTS OF CANNABINOIDS ON G-PROTEINS AND OTHER SIGNAL TRANSDUCTION COMPONENTS

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The CB<sub>1</sub> cannabinoid receptor is a member of the family of G protein coupled receptors. CB<sub>1</sub> receptor interactions with the Gi/o family have been defined by the ability of pertussis toxin to block a number of well-characterized signal transduction pathways (see 1, 2 for reviews). Widely recognized effector proteins include adenylyl cyclase and ion channels. Other signal transduction pathways, such as those involving mitogen-activated protein kinase (MAPK), focal adhesion kinase, and nitric oxide, have been reported.

The cytoplasmic surface of the CB<sub>1</sub> receptor and its ability to associate with and activate G proteins has been examined using peptides that represent defined regions of the 3<sup>rd</sup> loop (CB<sub>1</sub>301, CB<sub>1</sub>316, CB<sub>1</sub>329) and the juxtamembrane C-terminal region (CB<sub>1</sub>401) (3,4). When incubated with rat brain membranes, the juxtamembrane C-terminal peptide CB<sub>1</sub>401 promoted GTPγ[<sup>35</sup>S] binding to G proteins. When incubated with N18TG2 membranes, CB<sub>1</sub>410 promoted inhibition of hormone- or forskolin-stimulated adenylyl cyclase. Combination of all three of the third intracellular loop peptides (IL3) increased GTPγ[<sup>35</sup>S] binding to G proteins. This can be interpreted to mean that the peptides mimic the receptor domains that recognize and activate G proteins (3,4).

The receptor could be solubilized using the non-ionic detergent CHAPS, which allows protein-protein interactions to exist even after solubilization (5). The CB<sub>1</sub> receptor could be immunoprecipitated from CHAPS-solubilized rat brain or N18TG2 neuroblastoma cell membranes using an antibody that recognizes the extracellular N-terminal, a region that is not involved in ligand binding or signal transduction (6-8). The receptor and its associated proteins were separated on SDS-urea-PAGE and Western blot analysis was performed. Antibodies to the CB<sub>1</sub> receptor and specific G protein α subunits were used for Western analysis. In order to demonstrate that the CB<sub>1</sub> receptor-Gα complex represents a function association, the membranes or the solubilized extract were treated with pertussis toxin A subunit, and then coimmunoprecipitated for Western analysis. The pertussis toxin treatment disrupted the protein-protein interaction as would have been predicted because of the ADP-ribosylation of the Gi/o proteins at the domain required for association with G protein coupled receptors (6,8). This demonstrates that the receptor-Gα association is in dynamic equilibrium both in CHAPS as well as in intact membranes.

If the CB<sub>1</sub>401 peptide were directly interacting with the Gα protein, then the peptide would compete with the CB<sub>1</sub> receptor for binding to G proteins in CHAPS solution. When the CB<sub>1</sub>401 peptide was incubated with the CHAPS extracts from rat brain or N18TG2 cell membranes, the peptide disrupted the interaction between the CB<sub>1</sub> receptor and Gαo and Gαi3 (7,8). The CB<sub>1</sub>401 peptide failed to disrupt the CB<sub>1</sub> receptor-Gαi1 or Gαi2 interactions. Rather, a combination of all three peptides comprising the third intracellular loop disrupted the CB<sub>1</sub> receptor association with Gαi1 or Gαi2 in both rat brain and N18TG2 membrane extracts. In these experiments, the third loop peptides were unable to compete with Gαo in rat brain extracts or Gαi3 in N18TG2 membrane extracts. These studies (7,8) demonstrate that the juxtamembrane C-terminal domain recognizes and regulates Gαo and Gαi3, whereas the third loop domain recognizes and regulates Gαi1 and Gαi2.

It is possible that agonists having different structural properties may dock into the receptor and thereby induce or stabilize somewhat different conformations of the receptor (9,10). These conformations may exhibit very subtle differences in the helical assembly. Alterations in helix 5 and 6 orientation would be likely to alter the third



intracellular loop. In contrast, modifications in helix 7 orientation would dominate changes in the juxtamembrane C-terminal domain. If "activation" of the receptor by the structurally diverse agonist ligands results in reorientation of helices in an agonist-specific manner, then G protein subtypes would be differentially affected. This was demonstrated by the finding that various agonists could alter the CB<sub>1</sub> receptor-G protein associations differentially. The aminoalkylindole agonist WIN55212-2 disrupted the association with all three G $\alpha$  proteins, but did so only partially. The cannabinoid agonist desacetyllevonantradol disrupted the association with G $\alpha$ i1 partially and G $\alpha$ i2 completely, but failed to alter the interaction with G $\alpha$ i3. The eicosanoid agonist methanandamide disrupted the association with G $\alpha$ i3 completely, but failed to disrupt the interactions with G $\alpha$ i1 or G $\alpha$ i2.

Because structurally diverse CB<sub>1</sub> receptor agonists can evoke a differential influence on receptor-Gi/o subtype associations, we can predict that different agonists would activate a particular signal transduction pathway with varying degrees of intrinsic efficacy, depending upon which G protein subtype dominantly regulates that pathway. This concept offers a novel interpretation for data that might indicate that structurally diverse CB<sub>1</sub> receptor agonists may have different abilities to evoke a specific signal transduction response.

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## **EFFECTS OF CANNABINOIDS ON INTRACELLULAR PROCESSES: MODULATION OF MODULATION**

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Cannabinoids, the principal psychoactive constituent of marijuana, produce a characteristic spectrum of behavioral actions. Recent and ongoing studies suggest the majority of these effects are mediated by CB<sub>1</sub> cannabinoid receptors. CB<sub>1</sub> receptors are G protein-coupled receptors (GPCR's) that have as their major intracellular targets adenylyl cyclase, calcium and potassium channels, and MAP kinase. As might be expected from their central role in signal transduction, CB<sub>1</sub> receptors themselves are subject to modulation by several processes. This talk will focus on mechanisms of CB<sub>1</sub> receptor desensitization and the role of homodimerization in CB<sub>1</sub> receptor signaling.

Repeated administration of delta-9-THC leads to the rapid development of tolerance in a number of model systems. Similarly, the signaling of CB1 receptors in simple systems also undergoes rapid desensitization. Using the *Xenopus* oocyte expression system and two microelectrode voltage clamps, we found that phosphorylation of two residues in the C-terminus of the CB1 receptor underlies this rapid desensitization. In parallel to their desensitization, CB1 receptors also undergo agonist-induced internalization. In contrast to desensitization, internalization requires the extreme C-terminus of the CB1 receptor. Thus, the domains determining desensitization and internalization of CB1 receptors are completely distinct, suggesting these two processes may subservise different physiological functions in the cell.

Evidence from a number of studies conducted over the past few years has demonstrated that GPCR's can exist as dimers (or higher order multimers) with themselves, with other GPCR's as well as with other transmembrane proteins such as ion channels. CB1 receptors follow this pattern. Using an antibody directed against the dimerized form of the CB1 receptor, we found that CB1 receptors exist as dimers in rodent and human brain. Furthermore, dimerized CB1 receptors appear to be preferentially internalized from the cell membrane. Thus, CB1 dimerization appears to play a major role in the regulation of CB1 signaling.

Taken together, these results support the notion that CB1 receptors are subject to multiple interacting levels of control as they transduce the effects of exogenous and endogenous cannabinoids in the neuron.

## THE ROLE OF KINASE MODULATION IN THE ANTINOCICEPTIVE TOLERANCE TO $\Delta^9$ -THC

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Under conditions of acute cannabinoid exposure, cannabinoid [CB1 and CB2] receptors are G-protein coupled to  $G_{i/o}$  proteins which, when activated by phosphorylation, inhibit the activity of adenylyl cyclase. Upon agonist binding, the  $\beta\gamma$  subunit disassociates from the  $\alpha$  subunit of the G-protein. The  $\alpha$  subunit inhibits adenylyl cyclase, while the  $\beta\gamma$  subunit has been linked to stimulation of other cellular events such as the activation of tyrosine kinases (TK). Tolerance can develop to the *in vivo* and *in vitro* pharmacological effects of cannabinoids via unknown mechanisms. Receptor internalization is a possible mechanism of tolerance. Studies indicate that the cannabinoid receptor is rapidly internalized following binding of an agonist. There is indirect evidence that a variety of kinases could be involved in the development of tolerance to cannabinoids. The focus of our studies was the determination of the kinases that play a role in the expression of tolerance to the cannabinoids.

We addressed our hypothesis by evaluating the role of several kinases in tolerance to  $\Delta^9$ -THC. We evaluated protein kinase A [PKA] using KT5720, a PKA inhibitor; protein kinase C [PKC] using bisindolylmaleimide I, HCl (bis), a PKC inhibitor; protein kinase G [PKG] using KT5823, a PKG inhibitor;  $\beta$ -ARK using low molecular weight heparin (LMWH), a  $\beta$ -ARK inhibitor; PI3K using LY294002, a PI3K inhibitor and PPI, a src family tyrosine kinase inhibitor. Our data indicate that selective kinases may be involved in cannabinoid tolerance.

ICR mice were rendered tolerant to  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC). The PKG inhibitor, KT5823, the  $\beta$ -ARK inhibitor, LMWH, the PI3K inhibitor, LY294002 and inhibition of PKC by bis had no effect on tolerance. Bis, at a higher dose, attenuated the antinociceptive effect of  $\Delta^9$ -THC in non-tolerant mice. PPI, the src family tyrosine kinase inhibitor, and KT5720, the PKA inhibitor, reversed  $\Delta^9$ -THC-induced tolerance. These data support a role for PKA and tyrosine kinase in phosphorylation events in  $\Delta^9$ -THC-tolerant mice.

In non-tolerant animals, acute administration of  $\Delta^9$ -THC-induced reduction in cAMP formation also decreases likelihood that cAMP-dependent protein kinase (PKA) will be activated. It was not surprising therefore that we observed that injection of the PKA inhibitor, KT-5720, did not affect the antinociceptive potency of  $\Delta^9$ -THC. These results suggest that adenylyl cyclase is not constitutively active in sites mediating antinociception in non-tolerant animals. However during tolerance, CB1 receptors lose the ability to inhibit adenylyl cyclase, either through desensitization or switching to Gs-protein stimulation. The intrathecal (i.t.) administration of KT5720, a PKA

inhibitor, at a dose of 2.7 $\mu$ g/mouse in 100% DMSO vehicle (i.t.) significantly ( $p < 0.05$ ) reversed  $\Delta^9$ -THC antinociceptive tolerance in a dose-dependent manner, as determined by the tail-flick test. There was a leftward shift of the dose-response curve for  $\Delta^9$ -THC. The ED50 in the  $\Delta^9$ -THC-tolerant mice was shifted from 80 (95% CLs 62 to 102) to 8.6  $\mu$ g/mouse (95% CLs 4.7 to 16) in the KT5720-treated mice. The lines were parallel and the potency ratio was 8.3. Thus, our data indicate that PKA becomes constitutively active in  $\Delta^9$ -THC-tolerant mice.

CB1 receptor activation of the  $\beta\gamma$  subunit of G-proteins can stimulate tyrosine kinases (TK). The  $\beta\gamma$  subunit activates Src tyrosine kinase. Src tyrosine kinase has been shown to activate Ras, which can activate mitogen-activated protein kinase (MAP kinase). It has been clearly demonstrated that CB1 receptor activation stimulates MAP kinase. The Src tyrosine kinase inhibitor PP1 had no effect on the antinociceptive potency of  $\Delta^9$ -THC. However, at a dose of 0.0001 $\mu$ g/mouse, in 100% DMSO vehicle administered intrathecally (i.t.), PP1 significantly ( $p < 0.05$ ) reversed  $\Delta^9$ -THC antinociceptive tolerance in mice. The 0.0001 $\mu$ g/mouse dose of PP1 alone was shown to be inactive in the tail-flick test in naïve mice and in the non-tolerant group. The reversal of tolerance to  $\Delta^9$ -THC in the THC-tolerant group strongly indicates that the src TK is constitutively active in THC tolerance. Src tyrosine kinases are one of a broad family of receptor and cytoplasmic tyrosine kinases. Our data do not rule out the possibility that other TKs are critical to the expression of tolerance to  $\Delta^9$ -THC.

In summary, at least two pathways ( $\alpha$  subunit,  $\beta\gamma$  subunit) stimulated by the CB1 receptor appear to affect several signal transduction pathways leading to changes in cellular phosphorylation by protein kinases. In addition, PKA and src TK appear to become constitutively activated by the chronic administration of and development of tolerance to  $\Delta^9$ -THC. Given that tolerance also develops to the endocannabinoids, it will be important to compare and contrast the exogenous and endogenous cannabinoid systems as to the modulation and plasticity evoked in kinase activity during chronic administration of the drugs.

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## SYMPOSIUM V

### MOLECULAR AND BEHAVIORAL MECHANISMS MEDIATING PSYCHOMOTOR STIMULANT ABUSE: VIEWS OF YOUNG INVESTIGATORS

*S.L. Collins and H.L. Kimmel, Chairpersons*

#### INTRODUCTION

The purpose of the present proposal was to organize a symposium comprised of young investigators in the field of drug abuse research. Specifically, we chose to focus on psychomotor stimulant abuse, given its prevalence in today's society. It is important to understand why humans use drugs like cocaine and methamphetamine, so that effective therapeutics can be developed to treat psychomotor stimulant abuse. To achieve this goal, research efforts must focus on the molecular and behavioral mechanisms of drug abuse. A combination of pharmacological and behavioral treatments may have greater success than each alone. The present symposium involves a range of topics on the molecular and behavioral mechanisms mediating psychomotor stimulant abuse from regulation of the dopamine transporter by dopaminergic drugs to the behavioral phenomenon of self-control and its influence on drug taking. Considering the enormous literature on the relationship between stimulant abuse liability and dopaminergic effects, molecular mechanisms of interest for the present symposium focuses on the dopamine system. So as not to neglect the importance of other systems in abuse liability of psychomotor stimulants, the affect of opioid compounds on cocaine self-administration and dopamine neurochemistry is discussed. Behavioral mechanisms focus on the reinforcing and discriminative stimulus effects of stimulant drugs and how environmental factors influence drug intake. The present symposium is comprised of innovative and creative young scientists who contribute to our understanding of the molecular and behavioral mechanisms of drug abuse.

#### REGULATION OF THE DOPAMINE TRANSPORTER IN THE RAT BRAIN

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Neurotransmitter uptake into the presynaptic terminal or glial cells is an important mechanism for terminating the action of synaptic neurotransmitters (Amara and Kuhar 1993). These proteins responsible for uptake are sites of action for many drugs with both therapeutic and abuse potential (Amara and Pacholczyk 1991, Giros and Caron 1993, Kuhar 1993, Reith *et al.* 1997). Exposure to drugs such as cocaine can alter DAT levels in humans (Staley *et al.* 1994; Little *et al.* 1998) and in animals (Farfel *et al.* 1992, Letchworth *et al.* 2001). Since DAT has several important functions, it is necessary to examine the localization and regulation of these transporter proteins.

The balance of protein production and degradation in the cell determines the level of DAT protein. Previously, we developed a method for measuring the production rate, the degradation rate constant, and the half-life of the rat dopamine transporter (DAT) protein using the irreversible DAT ligand, RTI-76 (3 $\beta$ -(3-*p*-chlorophenyl) tropan-2 $\beta$  carboxylic acid *p*-isothiocyanatophenyl-ethyl ester hydrochloride) (Kimmel *et al.* 2000). In that study, we determined the half-life of the DAT protein in the rat striatum is about 2 days.

In the present studies, male Sprague-Dawley rats were treated by systemic drug injections once a day for three consecutive days. On the fourth day, rats were given a unilateral injection of saline or 100 nmol RTI-76 in the right lateral ventricle. Following recovery from surgery, all animals received their respective systemic saline or drug treatment every day thereafter until their sacrifice. Animals were sacrificed at 1, 2, 3, and 7 days after ICV injection of RTI-76 or saline. Levels of DAT protein in the striatum were determined by [<sup>3</sup>H]GBR12935 binding to homogenized striatal tissue. None of the drug treatments altered DAT levels in the striatum of the animals that received ICV saline, so these data are not shown. The half-life of recovery of [<sup>3</sup>H]GBR12935 binding of DAT in the striatum following inactivation by RTI-76 was determined by a monoexponential repopulation equation,

assuming a zero-order transporter production rate and a first-order transporter degradation rate constant (Mauger *et al.* 1982, Sladeczek and Bockaert 1983).

Since many of the behavioral effects of cocaine have been attributed to its actions at DAT, we examined the effect of systemically administered cocaine on DAT kinetics in the rat striatum. We found that the degradation rate constant and the production rate were decreased following cocaine treatment, resulting in a longer half-life of DAT in these animals (Table 1).

Systemic drug treatment	$t_{1/2}$ (days)	$k$ days <sup>-1</sup>	$r$ fm/mg/day
<b>Saline</b>	2.3	0.30	171
Cocaine (20 mg/kg, IP)	2.8	0.25	144

Table 1. DAT half-life ( $t_{1/2}$ ), degradation rate constant ( $k$ ), and production rate ( $r$ ) were determined following systemic treatment with saline or cocaine.

In order to determine how cocaine alters DAT kinetics, we focused on dopamine receptors as cocaine increases synaptic levels of dopamine, thereby increasing the stimulation of pre- and post-synaptic dopamine receptors. We administered the dopamine D1-like receptor agonist, SKF38393, the dopamine D1-like receptor antagonist, SCH23390, the dopamine D2-like receptor agonists, quinpirole, and R(-)-propylnorapomorphine hydrochloride (NPA), or the dopamine D2-like receptor antagonist, eticlopride systemically to rats, then determined DAT protein kinetics. Neither the D1 agonist nor the antagonist altered DAT kinetics. However, both D2 agonists increased the degradation rate constant and the production rate, decreasing the DAT half-life. The D2 antagonist decreased the degradation constant and the production rate, increasing the DAT half-life. The effects of the D2 agonist were blocked by a pretreatment with the D2 antagonist (Table 2).

Systemic drug treatment	$t_{1/2}$ (days)	$k$ days <sup>-1</sup>	$r$ fm/mg/day
Saline	1.9	0.36	217
SKF38393 (3.0 mg/kg, SC)	1.9	0.37	222
Saline	2.1	0.33	198
SCH23390 (0.5 mg/kg, IP)	2.0	0.35	201
Saline	2.0	0.35	212
NPA (0.1 mg/kg, IP)	1.4	0.48	296
Saline	2.0	0.35	213
Quinpirole (0.3 mg/kg, IP)	1.1	0.61	357
Saline	2.1	0.33	197
Eticlopride (0.5 mg/kg, IP)	2.6	0.27	169
Saline	2.0	0.34	207
Eticlopride (0.5 mg/kg, IP) + quinpirole (0.3 mg/kg, IP)	2.4	0.29	171

Table 2. DAT half-life ( $t_{1/2}$ ), degradation rate constant ( $k$ ), and production rate ( $r$ ) were determined following systemic treatment with saline, SKF38393, SCH23390, NPA, quinpirole, eticlopride, or eticlopride + quinpirole.

Thus, the systemic administration of dopamine D2 agonists and antagonists altered DAT kinetics in the rat striatum while the administration of dopamine D1 agonists and antagonists did not. The influence of D2 receptor ligands on DAT turnover may be partially explained by the co-localization of D2 receptors with DAT on the presynaptic membrane. In the striatum of the rat, DAT is localized in the plasma membrane of axons and terminals, along with D2 dopamine receptors (Hersch *et al.* 1997). Within the striatum, the majority of D1 and D2 receptors are found post-synaptically, although D2 receptors are also found presynaptically in dopamine neurons, where they function as autoreceptors (Levey *et al.* 1993, Sesack *et al.* 1994, Yung *et al.* 1995).

In support of the data in the present study, several recent studies show that dopamine D2 receptor activation and inhibition alter DAT function. *In vitro* rotating disk electrode voltammetry studies using rat striatal synaptosomal

suspensions showed that acute administration of the dopamine D2 receptor agonist, quinpirole, increased dopamine uptake (Meiergerd *et al.* 1993). Local application of the dopamine D2 receptor antagonist raclopride inhibited dopamine uptake in the rat striatum (Cass and Gerhardt 1994). The absence of dopamine receptors can also influence DAT function, as shown by dopamine D2 receptor-deficient mice, which exhibit decreased striatal DAT uptake (Dickinson *et al.* 1999).

Although the data with the dopamine receptor selective agonists and antagonists were consistent in this study, they do not explain the increase in the half-life observed after cocaine treatment. If cocaine increases dopamine levels, the increased stimulation of dopamine D2 receptors would presumably decrease DAT half-life, which contradicts our observations. Although we examined the effects of dopamine D1 and D2 receptor agonists on DAT kinetics, these agonists were administered separately. The simultaneous administration of these agonists may emulate the effects of the cocaine-induced increases in synaptic dopamine on dopamine receptors more accurately. As cocaine binds to other monoamine transporters, the effects of DAT-selective compounds on DAT turnover should be investigated. Cocaine has a shorter duration of action than some of the receptor-selective compounds do, so the effects of shorter- and longer-acting compounds on DAT turnover should be determined.

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## **CHRONIC OPIATE ADMINISTRATION ALTERS COCAINE BEHAVIORS AND DOPAMINERGIC NEUROCHEMISTRY**

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It has been shown previously that chronic cocaine increases kappa-opioid receptor density (Unterwald *et al.* 1994) and increases prodynorphin gene expression in the rat striatum (Daunais *et al.* 1993, Spangler *et al.* 1993, Romualdi *et al.* 1996). However, since cocaine inhibits the reuptake of dopamine, norepinephrine, and serotonin, it is not clear which neurochemical systems underlie the alteration of prodynorphin mRNA by cocaine. The pattern of alterations in prodynorphin gene expression by the selective dopamine uptake inhibitor GBR 12909 differs from that of cocaine (Romualdi *et al.* 2001), suggesting that this interaction is not regulated solely by dopamine.

Alternatively, kappa-opioid receptor agonists diminish the behavioral activating effects of cocaine (Heidbreder *et al.* 1993, Schenk *et al.* 1999, Collins *et al.* 2001, in press) and repeated administration of the kappa-opioid receptor agonist U-69593 produces an increase in extracellular dopamine levels (Heidbreder *et al.* 1998) and a marked reduction in dopamine D<sub>2</sub> receptors in the caudate putamen (Izenwasser *et al.* 1998). This suggests that regulation of dopamine neurotransmission by the kappa-opioid receptor may provide a mechanism by which the effects of cocaine can be altered.

To better understand the interactions between brain opioid and dopamine systems and the role that they might play in stimulant abuse, the effects of cocaine and selective dopamine uptake inhibitors were examined on prodynorphin mRNA and kappa-opioid receptor binding. In addition, the effects of the selective kappa-opioid receptor agonists U-69593 or bremazocine were examined on cocaine-induced locomotor activity and the neurochemical substrates in brain circuits that mediate the behavioral effects of cocaine.

Rats were continuously infused for one week with cocaine or the selective dopamine uptake inhibitor GBR 12909 or injected daily for one week with GBR 12909. Dynorphin, the endogenous ligand for the kappa-opioid receptor, was measured following the treatment phase. Prodynorphin mRNA was increased in the caudate putamen of rats treated with cocaine but not following GBR 12909 infusion or daily injection. Because the selective dopamine uptake inhibitor had no effect on dynorphin in the caudate putamen, but cocaine, which inhibits dopamine, serotonin and norepinephrine, increased dynorphin, this suggests that a system other than dopaminergic may be regulating the interaction between cocaine and the kappa-opioid system. Furthermore, chronic administration of selective dopamine uptake inhibitors have no effect on kappa-opioid receptor binding, while chronic cocaine increased kappa-opioid receptor binding in brain regions that are highly enervated by serotonin, suggesting that the interaction between cocaine and the kappa-opioid system may involve the serotonergic system.

To examine the effect of kappa-opioid receptor agonists on cocaine-related behaviors, rats were injected once daily for five days with the  $\kappa$ -opioid receptor agonist U-69593 or vehicle and locomotor activity was measured. Daily treatment with U-69593 decreased activity levels compared to rats treated daily with vehicle. Three days later, rats were administered cocaine or saline daily for the next five days (days 8-12). Previous treatment with U-69593 blocked cocaine-induced activity levels compared to rats treated with vehicle then given cocaine. One week later, on day 19, rats were challenged with cocaine or saline. In rats previously given vehicle, the cocaine challenge produced a sensitized response that was not observed in rats previously given U-69593 (Collins *et al.* 2001, in press).

In a separate experiment, rats were injected once daily for five days with U-69593, the  $\kappa$ -opioid receptor agonist bremazocine, or vehicle in combination with cocaine, to determine the effects of kappa-opioid receptor agonists on long-term cocaine use. Rats administered vehicle + cocaine showed an increase in activity levels that was not seen in either group administered a kappa-opioid receptor agonist + cocaine. A cocaine challenge was then administered on both day 8 and day 15. Vehicle + cocaine-treated rats were sensitized to the cocaine challenge. Neither group treated with a kappa-opioid receptor agonist showed sensitization to either cocaine challenge (Collins *et al.* 2001, in press).

An interval treatment regimen of U-69593 was studied where U-69593 or vehicle was administered every 3 days over a 15-day period. The decrease in activity levels observed with daily treatment of U-69593 was not observed in rats that were treated with U-69593 every third day. However, these rats still exhibited significantly decreased cocaine-induced locomotor activity when compared to rats treated with vehicle (Collins *et al.* in press).

To understand which system is regulating the effects of kappa-opioid receptor agonists on cocaine-related behavior, neurochemical assays were conducted in rats that were treated for five days (once daily) with a kappa-opioid receptor agonist alone or in combination with cocaine, or treated every 3 days for 15 days with a kappa-opioid receptor agonist, and killed three days later. Kappa-opioid receptor agonist treatment alone decreased dopamine transporter density and increased levels of tyrosine hydroxylase. However, there was no effect of kappa-opioid receptor agonist + cocaine treatment, or of treatment with a kappa-opioid receptor agonist every 3 days for 15 days, on dopamine transporter density or dopamine D<sub>2</sub> receptor density. There was no effect of any kappa-opioid receptor agonist treatment alone or in combination with cocaine on dopamine D<sub>1</sub> receptor density (Collins *et al.* 2001, in press).

Thus, it is possible that while kappa-opioid receptor agonists may block cocaine-induced locomotor activity via an interaction with brain dopamine systems, another system, possibly the serotonergic system, may also play a role in the marked decrease in cocaine-induced behaviors following kappa-opioid receptor agonist treatment. Furthermore, the lack of effect of selective dopamine uptake inhibitors on dynorphin levels and kappa-opioid receptor binding, in contrast to the increase observed following cocaine treatment, suggests that the interaction between cocaine and kappa-opioid receptors may be regulated by systems other than dopaminergic. This is supported by the change in kappa-opioid receptor binding that occurred in regions enervated by serotonin and not dopamine following cocaine treatment. Overall, these findings show that the kappa-opioid system and cocaine interact in a reciprocal manner and that this interaction may be regulated, at least in part, by the serotonergic system. Supported by DA 11960.

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## EFFECTS OF DOSE AND INFUSION DELAY ON COCAINE SELF-ADMINISTRATION CHOICE IN RHESUS MONKEYS

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An individual's choice between different alternatives is determined by many variables, including reinforcer magnitude and delay to reinforcer presentation. The interaction of these variables and their effects on choice have been studied with human subjects and monetary outcomes (e.g., a choice between \$1000 in one week or \$100 now) and with non-human subjects and food (e.g., three food pellets later versus one pellet now). Specific arrangements of these contingencies have led to models of impulsivity, a behavior pattern that is often correlated with substance abuse. Impulsivity is operationally defined as a choice for a smaller, more immediate reinforcer over a larger, more delayed reinforcer. Self-control is the converse, a choice for a larger, more delayed reinforcer over a smaller, more immediate reinforcer. An understanding of the factors involved in impulsive choice may have relevance to the prevention and treatment of drug abuse. However, no published study to date has examined such choices in a drug self-administration context. The present study was designed to investigate the interaction between dose and delay on rhesus monkeys' choice maintained by cocaine self-administration.

Five male rhesus monkeys implanted with intravenous double-lumen catheters served as subjects. They were housed individually in experimental cubicles that were equipped with two response levers, each with a set of stimulus lights. A discrete-trials choice procedure was used and sessions were divided into two blocks of ten trials each. The first two trials of each block were forced-choice trials and the following eight were free-choice trials. During forced-choice trials, only one alternative was available at a time and white lever lights were illuminated above the active lever. The first forced-choice trial was determined randomly and was followed by the second (alternate) forced-choice trial. Thus, the subjects received exposure to both sets of contingencies before being allowed to choose between them in the free-choice trials. During free-choice trials, both alternatives were presented simultaneously and the subject's choice was recorded. Cocaine infusion was contingent upon a single lever press (fixed-ratio 1 schedule of reinforcement). The lever press initiated a delay period that was followed by a 10-s infusion of cocaine. The infusion was paired with a change in stimulus lights, either from white to orange or from white to blue, depending on the selection. The colored lights remained on constantly during the infusion, but flashed during the preceding delay period. Drug presentation was followed by a timeout or intertrial interval that was 30 minutes minus the delay period. This ensured that trials would begin every 30 minutes regardless of the outcome selected. Drug doses and delay periods were always presented in a 3:1 ratio. Thus, the relative doses and delays remained constant throughout the experiment, but the absolute values were altered. All data were taken from the 16 free-choice trials in the two blocks.

The experiment was divided into four phases. In the first phase, the delay to cocaine presentation was equal (30 s), regardless of lever selection, and the dose of cocaine was manipulated across two conditions. In one condition, cocaine choice was between 0.3 versus 0.1 mg/kg/inj and in the other condition, choice was between 0.1 and 0.03 mg/kg/inj. In the second phase, the dose of cocaine was held constant (0.1 mg/kg/inj) and the delay to presentation was manipulated across four conditions: 30 s versus 10 s, 90 s versus 30 s, 270 s versus 90 s, and 810 s versus 270 s. In the third and fourth phase, dose and delay were both manipulated so that choice would match the operational definitions for self-control and impulsivity. The outcomes consisted of a low dose of cocaine presented after shorter delay (the impulsive choice) or high dose presented after longer delay (the self-control choice). In the third phase, the lower dose combination (0.1 versus 0.03 mg/kg/inj) was evaluated with all four delay combinations. In the fourth phase, the higher dose combination (0.3 versus 0.1 mg/kg/inj) was studied with all four delay combinations. Lever reversals were implemented in all phases and all conditions of the experiment.

Data from the first phase (equal delays) showed that subjects' choices were nearly exclusive for the larger dose in both conditions. Data from the second phase (equal dose) showed that choice was nearly exclusive for the alternative associated with the shorter delay in all four conditions. Choice in phases three and four indicated that as the absolute value of the delays increased, preference reversed from the self-control option to the impulsive option, i.e., the number of large-dose choices decreased as a function of increasing delays to drug presentation. This preference reversal occurred with the relative values of the delays held constant.

Thus, the data support the notion of delay discounting, which states that the value of a reinforcer is decreased or discounted by its delay to presentation. More specifically, the data support the hyperbolic delay-discounting model in that preference reversals were observed. However, the reversals noted here are in contrast with the predictions of the hyperbolic delay-discounting model, which suggests that preference should reverse from impulsive choice at short delays to more self-controlled choice when the delays are long. This contrasting finding could be a result of the species tested, the nature of the reinforcer, or procedural variables, e.g., delay values, conditioned reinforcement mediating the delay period. Future studies will be designed to further investigate environmental and pharmacological factors that determine choice in an animal model of impulsive drug taking.

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## SYMPOSIUM VI

### CONSEQUENCES OF PSYCHOMOTOR STIMULANT ABUSE: PET IMAGING IN MONKEYS

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The aim of this symposium was to describe recent brain imaging studies using positron emission tomography (PET) in various non-human primate models of psychomotor stimulant abuse. The use of PET allows for the systematic study of brain function as a consequence of drug exposure, withdrawal, cue-induced craving and relapse. Monkeys provide a unique model to study long-term consequences of drug exposure and the studies described in this symposium used longitudinal designs over years to systematically examine changes in several CNS markers as a consequence of drug exposure and following changes in environmental context. Overall, these studies in monkeys, using PET imaging technology and sophisticated behavioral methods, will help identify what brain areas mediate psychostimulant reinforcement, which will ultimately facilitate the development of effective pharmacotherapies.

#### METHAMPHETAMINE-INDUCED NIGROSTRIATAL DOPAMINE SYSTEM DEFICITS IN MONKEYS: EXTRAPOLATION TO HUMANS

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As methamphetamine (METH) abuse has become more widespread over the past decade, it is critical to elucidate the neurobiological mechanisms associated with its long-term abuse. The central question that arises from the wealth of recent research data from animal studies is to what extent it can provide an accurate interpretation of the effects of METH in humans. In this presentation, aspects of METH pharmacology that are associated with its potential neurotoxicity are addressed. Specifically, examples of METH toxicity in non-human primates are followed by recent data on the neurotoxicity of human METH exposure.

The term neurotoxicity is often used to indicate neurodegeneration, that is, the irreversible loss of nerve terminals or neuron cell bodies. Herein, a more inclusive definition of neurotoxicity is used as that formulated by The Interagency Committee on Neurotoxicology: "Neurotoxicity is any adverse effect on the structure or function of the central and/or peripheral nervous system by a biological, chemical or physical agent. Neurotoxic effects may be permanent or reversible, produced by neuropharmacological or neurodegenerative properties of a neurotoxicant, or the result of direct or indirect actions on the nervous system" (Erinoff, 1995). In this overview, the neurotoxic effects of METH on the striatal dopamine (DA) system are described.

The neurotoxicity observed in the striatal DA system has been characterized in several animal models, most extensively in the rodent and less so in the monkey (Seiden, 1991). In rat studies, METH exposure results in long-term decreases in striatal DA uptake binding sites associated with the DA transporter (DAT), in DA concentrations, and of DA system-related proteins, tyrosine hydroxylase (TH), and the vesicular monoamine transporter (VMAT). Additionally, after "high dose" METH, as well as with MDMA, alterations in the diameter and density of neuronal fibers and evidence of nerve fiber/terminal degeneration have been observed (Ricaurte *et al.* 1982). In non-human primates studies, extensive METH-induced DA decreases in DAT, VMAT binding, DA synthesis rates, and DA concentrations have also been observed. However, long-term studies have noted that these apparent neurotoxic effects are reversible to variable extents (Melega *et al.* 1997; Harvey *et al.* 2000). Both the magnitude of the striatal dopamine system neurotoxicity and its recovery over time have been demonstrated, *in vivo*, in monkey studies with positron emission tomography (PET) as shown in the examples below.

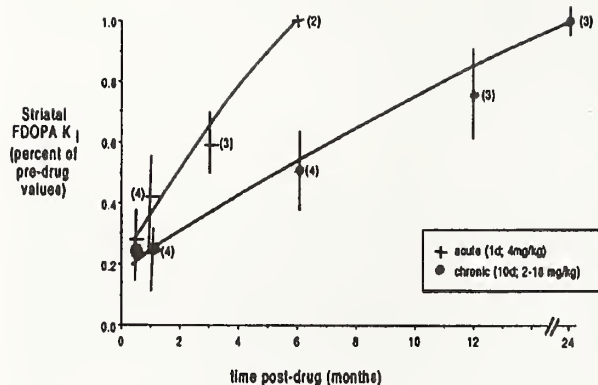


Figure 1



Figure 2

**Figure 1:** The time course for recovery of DA synthesis capacity in striatum in individual subjects is assessed with [<sup>18</sup>F]FDOPA – PET for two dosage regimens of *d*-amphetamine. The acute treatment consisted of 1 day of 4 mg/kg, im; the chronic treatment consisted of 10 days of escalating doses, extending from 2 to 18 mg/kg/day, im.

**Figure 2:** The time course for recovery of DAT binding density in striatum as assessed with [<sup>11</sup>C]WIN 35,428 - PET is shown for a dosage regimen of *d*-methamphetamine with 2 doses of 2 mg/kg, im, 6h apart. The image on the left is from a referent monkey. Additionally, a METH subject is shown at two time points: the WIN influx rate constant (K<sub>i</sub>) was reduced by 80% at 1 week (middle) and by <10% at 1.5 years (right) relative to referent values.

The magnitude of these changes is not associated with DA cell body loss in the substantia nigra. The persistent integrity of the cell bodies may underlie the remarkable reversibility of the significant decreases in indices of DA system integrity. However, because the extent of the losses are so severe (>80%) and the recovery essentially complete, the degeneration-regeneration hypothesis may not fully explain these data. We have proposed, alternatively, that phenotype suppression without axonal degeneration may contribute to these profiles of observed deficits. This conclusion is more apparent in the DA cell bodies where a similar reduction in DA indices is observed. That is, those reductions occur in cell bodies that ultimately survive the METH-induced toxicity, thereby eliminating the possibility of a degeneration-regeneration occurrence to explain that recovery. Further studies are presently being conducted to establish the actual extent to which degeneration does occur in this animal model.

The modeling of human METH abuse patterns is complicated by the lack of accurate data, especially when it is derived anecdotally from reports by drug abusers. However, a generic METH profile includes a transition from a pattern of “recreational” use and frequency to one of incremental doses in multiple “hits” over shorter periods of time, culminating in “binges” (Cho *et al.* 2001). The consequences of this gradual escalation in METH dosage and frequency may be different from animal models that do not parallel this type of METH administration regimen. Thus, a human binge pattern may not result in the kind or degree of toxic effects often associated with acute high METH doses in animal studies because of the apparent tolerance developed in humans to many of METH’s actions. Nonetheless, several of those METH-induced striatal alterations as characterized previously in non-human primate studies have now been shown to occur in humans. Specifically, DA concentrations (suggestive of reduced DA synthesis capacity) were recently reported in post-mortem in brains of human METH abusers (Wilson *et al.* 1996). Those deficits were not attributed to nerve terminal loss because another index of neuronal integrity, VMAT, was not correspondingly reduced, implying preservation of neuronal vesicles and the terminals that contained them.

More recently, PET imaging studies in human METH abusers have shown 20-30% decreases in the binding of PET radioligands to the DAT (McCann *et al.* 1998; Volkow *et al.* 2001). The functional significance of these alterations remains to be clarified; however, the results provide further evidence that METH-induced neuroadaptations occur in human. With that database, more appropriate animal models can now be designed to mimic the human condition.

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## USE OF PET IMAGING TO CHARACTERIZE THE NEUROPHARMACOLOGY OF COCAINE IN NON-HUMAN PRIMATES

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Positron emission tomography (PET) imaging techniques were used in awake rhesus monkeys as a novel approach to investigate cocaine-induced functional changes in CNS activity. The studies described used a customized head holder constructed of imaging-compatible materials (polycarbonate, polypropylene and hard rubber) to prevent movement during the scan acquisition. While the monkey was lightly anesthetized with ketamine, a fast-drying plaster wrap was used to produce a near-exact replica of each subject's head. Subsequently, the replica was enclosed within a Lexan helmet, and commercially-available, non-toxic insulation foam (polymeric diisocyanate and polyol resin) filled the void volume and hardened rapidly within the helmet. During PET imaging experiments, the subject's head was enclosed within the foam mold and Lexan helmet with adequate ventilation provided by an opening around the nose and mouth. The head-restraint device was attached to a molded plastic form that effectively restrained the limbs and torso in order to minimize body movements. The entire device easily attached to a standard Primate Products chair to facilitate immobilization. Once the animal had been placed in the specially-designed primate chair and effectively restrained, the restraint device was removed, transported to the Emory PET Center, and attached to the end of the scanning table to allow for proper orientation in the tomograph.

Functional changes in regional cerebral blood flow (rCBF) were characterized in four rhesus monkeys with the positron-emitting tracer <sup>15</sup>O water following acute i.v. administration of cocaine (0.3 and 1.0 mg/kg). Regions of interest were defined on MRI scans and then transformed into the coordinate system of the PET scans with a high degree of accuracy. Cocaine had pronounced, dose-related effects on blood flow at 5 min post-injection that diminished markedly by 25 min post-injection. The time course of effect on rCBF closely paralleled that reported for subjective measures of cocaine-induced euphoria in humans. Regions of interest showing significant increases in blood flow included whole brain, frontal, striatal and medial temporal regions. Brain activation maps normalized to global flow showed prominent cocaine-induced activation of prefrontal cortex that was dose-dependent and diminished markedly by 25 min post-injection. Importantly, cocaine-induced activation of blood flow in prefrontal

cortex was markedly attenuated following pretreatment with the SSRI, alaproclate. The latter results document a distinct pattern of brain activation following cocaine administration, and the ability of pharmacological pretreatment to alter cocaine-induced brain activation. Administration of alaproclate alone had no significant effect on global flow, and cocaine-induced changes in global flow were not affected by alaproclate, suggesting that the interactions between cocaine and alaproclate on rCBF were not simply related to direct cardiovascular effects. The dose of alaproclate that was effective in reducing cocaine-induced brain activation also attenuated the behavioral-stimulant and reinforcing effects of cocaine. Moreover, alaproclate pretreatment dose-dependently attenuated cocaine-induced increases in extracellular dopamine (DA) determined by *in vivo* microdialysis in awake squirrel monkeys. These results are consistent with a growing literature demonstrating that serotonin can effectively modulate the behavioral effects of psychomotor stimulants in an inhibitory manner. Concordant results obtained with PET neuroimaging, behavioral and neurochemical studies demonstrate a close association between drug-induced functional changes in rCBF and the reinforcing effects of cocaine. Accordingly, the pattern of brain activation induced by acute administration of cocaine may provide a technical and theoretical framework for screening pharmacotherapies in the treatment of cocaine addiction.

We extended our neuroimaging studies to characterize cocaine-induced changes in rCBF during active drug self-administration protocols. The PET apparatus was modified to include an array of stimulus lights and access to a response lever. Subjects were trained to respond under a fixed-ratio schedule of intravenous injections of cocaine in the presence of a red light, and the stimulus lights changed to white during drug infusions. Appropriate stimulus control of self-administration was established, as evidenced by a lack of responding in the absence of the red light. Compared to non-contingent cocaine administration, self-administered cocaine had more pronounced and longer-lasting effects. Importantly, the pattern of brain activation differed markedly under the two conditions. Areas of major activation included anterior cingulate and insular cortex, two regions associated with the extended limbic system. There was also a major cerebellar activation that was completely lacking in the non-contingent protocol. These initial experiments document the successful development of PET imaging protocols in behaving monkeys and the importance of response-contingent drug administration paradigms. Clearly, the pattern of brain activation during voluntary drug use in a specific context differs from that induced by the direct pharmacological effects of cocaine. Ongoing studies will determine the pattern of brain activation induced by drug-associated stimuli in the absence of cocaine.

In related studies, a new PET ligand was validated for quantitatively mapping the DA transporter (DAT). FECNT is a nortropane derivative and a selective ligand with nanomolar affinity for the DAT. It exhibits a 25-fold and 150-fold selectivity for the DAT relative to the serotonin and norepinephrine transporters, respectively. A technique was devised for radiolabeling [ $^{18}\text{F}$ ]FECNT, and its regional distribution was characterized in the rhesus monkey. [ $^{18}\text{F}$ ]FECNT exhibited high uptake in the caudate and putamen, and very little uptake in the hypothalamus/midbrain, cortex and cerebellum. The ratio of striatum to cerebellum was 6:1 at 125 min. [ $^{18}\text{F}$ ]FECNT reached a peak uptake in the caudate and putamen at 60 and 75 min post-injection, respectively, and time activity curves for the caudate, putamen, cortex and cerebellum indicated that binding site equilibrium was attained. Importantly, an *in vivo* displacement study of [ $^{18}\text{F}$ ]FECNT by cocaine was performed to determine whether striatal radioactivity reflected [ $^{18}\text{F}$ ]FECNT binding to the same affinity site of the DAT as cocaine. Dose-dependent *i.v.* chases of unlabeled cocaine were administered after injection of [ $^{18}\text{F}$ ]FECNT. Doses of cocaine (0.1 and 1.0 mg/kg) that are reliably self-administered by rhesus monkeys displaced [ $^{18}\text{F}$ ]FECNT binding by  $53 \pm 5$  and  $87 \pm 5$  %, respectively. The results demonstrate that drug-induced displacement of [ $^{18}\text{F}$ ]FECNT can be used to quantify the percent occupancy of the DAT by cocaine and by medication pretreatments that target the DAT. Subsequent studies compared DAT occupancy by selective DAT inhibitors to their effectiveness in reducing cocaine use. It was our contention that transporter occupancy measures will provide critical information concerning the selection of rational dosing regimens in clinical studies. Once validated, the conceptual framework and technical approach can be extended directly into human studies of cocaine addiction.

We extended our research with selective DAT inhibitors into rhesus monkey studies to evaluate further their reinforcing effects and their effectiveness in reducing cocaine self-administration. Eight rhesus monkeys were trained to self-administer cocaine (0.03-1.0 mg/kg) under a second-order schedule of *i.v.* drug delivery. The DAT inhibitors studied in substitution and pretreatment experiments included the phenyltropane derivatives, RTI-113 and RTI-177, and the phenylpiperazine derivative, GBR 12909. Importantly, we determined DAT occupancy with PET at the maximum reinforcing doses of cocaine and the DAT inhibitors, and at doses of the DAT inhibitors that

decreased cocaine-maintained response rates. The second-order schedule was well suited for drug-interaction experiments because response rate was a direct function of unit dose administered. Pretreatment with each of the DAT inhibitors dose-dependently decreased cocaine self-administration at two different maintenance doses of cocaine. PET imaging studies in the same subjects documented that high levels of DAT occupancy were required to reduce cocaine self-administration. Effective pretreatment doses for each of the DAT inhibitors required >80% DAT occupancy. *In vivo* microdialysis studies in awake squirrel monkeys also showed that effective pretreatment doses produced significant increases in extracellular dopamine. Ineffective pretreatment doses yielded DAT occupancy measures as high as 70%.

The high DAT occupancy measures obtained suggested that effective pretreatment doses of the DAT inhibitors may exhibit reinforcing properties when tested in drug self-administration protocols. Accordingly, drug substitution procedures were implemented to characterize the abuse liability of the DAT inhibitors when substituted for cocaine. When the DAT inhibitors were substituted for cocaine, at least one dose maintained self-administration above saline levels in all monkeys tested. However, maximum rates of responding maintained by the DAT inhibitors were less than those obtained for cocaine over a wide range of doses, suggesting that the DAT inhibitors were less effective in maintaining self-administration. Doses of cocaine that maintained maximum rates of responding yielded between 65-75% DAT occupancy, consistent with recent human studies reported by Volkow and colleagues. Importantly, doses of the DAT-selective inhibitors that maintained maximum rates of responding less than cocaine yielded >95% DAT occupancy. These results support the view that selective DAT inhibitors may be developed that effectively reduce cocaine self-administration while having reduced abuse liability compared to cocaine. Pharmacokinetic considerations that result in slow onset and long duration of action may be critical in limiting the abuse liability of candidate medications. Overall, these preclinical studies demonstrate the importance of combining self-administration studies with brain imaging techniques in monkeys in order to better understand cocaine's mechanisms of action. Ultimately, this approach should assist in the identification of potential cocaine pharmacotherapies.

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## **THE USE OF PET IMAGING TO EXAMINE THE EFFECTS OF COCAINE AND ENVIRONMENTAL CONTEXT ON DOPAMINE D<sub>2</sub> RECEPTORS IN MONKEYS**

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The overriding goal of this research is to broaden our understanding of the neural and behavioral mechanisms that mediate the reinforcing effects of cocaine. More specifically, these studies combine behavioral models of cocaine abuse in monkeys with the brain imaging procedure positron emission tomography (PET), in an effort to better understand the neuropharmacological effects of cocaine. It is well established that cocaine's reinforcing effects are mediated, in large part, through dopamine (DA) neurotransmission. Several PET studies in humans have confirmed a prominent role of DA receptors, in particular D<sub>2</sub> receptors, in mediating the reinforcing effects of cocaine (e.g., Volkow *et al.* 1990). Our laboratory has extended these findings to non-human primates self-administering cocaine for long periods of time and over extended abstinence periods, in order to better understand the role of D<sub>2</sub> receptors in mediating cocaine self-administration. A particular advantage of using non-human subjects to study changes within the brain is the absolute control over past and current drug use. Thus, we can measure D<sub>2</sub> receptor function in cocaine-naïve animals and examine how that system changes in response to acute and chronic cocaine reinforcement. In addition, we can systematically modify the environment in an effort to understand how environmental context changes brain function and subsequently the reinforcing effects of cocaine. Such experimental situations should allow for the assessment of variables related to vulnerability, as well as determine when receptor changes are long lasting.

The overall premises of the studies described, using various monkey models of cocaine self-administration and PET imaging of DA D<sub>2</sub> receptors, are that: (1) basal D<sub>2</sub> receptor levels are associated with a vulnerable individual; (2) cocaine can produce long-lasting changes in D<sub>2</sub> receptor density; (3) non-drug reinforced behaviors may be a "sensitive" marker of a vulnerable phenotype; and (4) environmental variables (in particular social rank) can also modify D<sub>2</sub> receptor levels which, in turn, can influence the reinforcing effects of cocaine.

To address points 1-3, twelve individually housed and experimentally naïve male rhesus monkeys were trained to respond under a multiple fixed-interval (FI) 3-min schedule of food presentation and baseline PET studies were conducted. Next, the conditions were changed to a multiple food, cocaine (0.2 mg/kg/inj) schedule and PET studies were repeated at various times over a 1 year period. In the behavioral sessions, food components lasted for 20 min or until 5 reinforcers were obtained and cocaine components were 60 min in duration or until 15 injections were delivered; each component cycled twice per session. PET scans were conducted in each monkey using the D<sub>2</sub>-selective radioligand [<sup>18</sup>F]fluorocleobopride (FCP). Previous studies from our group have validated this ligand for use in non-human primate studies (e.g., Mach *et al.* 1996; Nader *et al.* 1999). To control for cocaine-induced elevations in DA, PET scans occurred 24 hrs after the last self-administration session; in addition, each monkey was administered lorazepam (1.0 mg/kg, i.v.) 30 min prior to the PET study.

Regarding point (1), baseline D<sub>2</sub> receptor binding potential correlated with mean rates of cocaine-maintained responding during weeks 3 through 10 of cocaine self-administration. The lower the D<sub>2</sub> receptor levels (determined in monkeys before they were given the opportunity to self-administer cocaine), the higher the rates of cocaine-maintained responding. This supports earlier work in humans suggesting that low levels of D<sub>2</sub> receptor numbers are associated with reinforcing effects of psychomotor stimulants (Volkow *et al.* 1999). Related to point (2) and using a within-subjects design, we found that reinforcing doses of cocaine decreased D<sub>2</sub> receptor binding potential by ~15% within 1 week of self-administration and that after 1 year of self-administration the decreases in D<sub>2</sub> receptor levels reached a maximum of ~22%. Tolerance did not develop to the rate-decreasing effects of self-administered cocaine on food-maintained performance. When five monkeys with a 1 year history of cocaine self-administration were studied in abstinence, D<sub>2</sub> receptor levels recovered in 3 monkeys within 3 months, but in 2 monkeys remained ~20% below baseline after 1 year of abstinence. Interestingly, and related to point (3), we found that the disruptive effects of cocaine on food-maintained responding predicted which monkeys would show recovery of D<sub>2</sub> receptor levels during abstinence.

To address point (4), 20 male cynomolgus monkeys were studied first while individually housed and then after social housing (see Morgan *et al.* 2001). No differences in D<sub>2</sub> receptor levels were observed between the monkeys while individually housed, but after 3 months of social housing, monkeys that became dominant had a ~20% increase in D<sub>2</sub> receptor binding potential, while D<sub>2</sub> levels did not change in subordinate monkeys. When cocaine was made available for self-administration under a fixed-ratio schedule, significant differences were observed in response rates and cocaine intake as a function of social rank. We found that in subordinate monkeys - animals with lower levels of D<sub>2</sub> receptors - cocaine maintained significantly higher rates of responding compared to cocaine self-administration by dominant monkeys. In fact, cocaine did not function as a reinforcer in dominant monkeys. These findings suggest that environmental context, in this case social housing and subsequent social rank, modified D<sub>2</sub> receptor numbers and that these changes were associated with differential vulnerability to cocaine abuse. Overall, these studies indicate that combining behavioral pharmacology with brain imaging can provide valuable information about the interactions between drugs, the environment and the organism. This approach should aid in the understanding of variables that mediate cocaine's high abuse liability, and ultimately in identifying effective behavioral and pharmacological treatment strategies.

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## **IMAGING STUDIES OF STIMULANT ABUSE: LINKING FINDINGS FROM NON-HUMAN PRIMATES TO THOSE IN HUMAN SUBJECTS**

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The findings from imaging studies of non-human primates in stimulant drug abuse have started to shed light on mechanisms underlying addiction and neurotoxicity that are directly pertinent to the abuse of stimulant drugs in human subjects. The following section links these findings to those encountered by imaging studies of stimulant drug abusers.

### **Neurotoxicity of brain dopamine (DA) systems in methamphetamine (METH) abusers**

The work by Melega and colleagues using PET to monitor changes in DA terminals after METH administration have provided evidence of significant and long lasting changes in DA transporters (DAT) and in DA synthesis in non-human primates and have shown that some of these changes may recover with protracted detoxification. Because the doses and patterns of METH administered to laboratory animals differ from those used by drug abusers, it has been unclear whether similar changes in brain DA activity as those reported to occur in animals occur in humans. The data in humans are rather limited and to our knowledge only four studies have been published; one is a postmortem study of twelve METH abusers (Wilson *et al.* 1996), and the others are three imaging studies one in six methamphetamine abusers (McCann *et al.* 1998), 1 in 15 METH abusers (Volkow *et al.* 2001) and 1 in 11 abusers (Sekine *et al.* 2001). These studies reported reductions in brain DAT, which suggests that methamphetamine at the doses abused by humans also affects the DA terminals. The DAT losses reported were significantly smaller (20-30%) than those reported in animal studies (> 50%) (Seiden and Sabol 1996). These differences between animals and humans could reflect the differences in doses, patterns of use, interspecies variability and/or co-administration of other drugs by humans (e.g., nicotine, which has been shown to be neuroprotective against METH-induced toxicity) (Maggio *et al.* 1998).

The DAT losses reported in the METH abusers were also smaller than those found in Parkinson's disease, where DAT reductions are proportional to disease severity and range between 36% and 71% (Innis *et al.* 1993; Asenbaum *et al.* 1997; Frost *et al.* 1993). However, it should be noted that the range of striatal DAT losses in the METH abusers varies between 0-50%, which indicates that in some METH abusers, the level of DAT may fall within the range seen in patients with mild severity Parkinson's disease. Though there are no reports of extrapyramidal symptoms in METH abusers, this may reflect the fact that these subjects are still relatively young and can compensate. Nonetheless, the DAT reductions in METH abusers were associated with impaired motor performance and impaired verbal learning; the lower the DAT levels the worse the performance (Volkow *et al.* 2001). A similar association, albeit for much more severe disruption, has been reported (using PET) in patients with Parkinson's disease and between motor performance and DA cell activity (using [<sup>18</sup>F]fluoro-L-DOPA; Vingerhoets *et al.* 1997) and between DAT levels and verbal learning (using [<sup>11</sup>C]nomifensine; Marie *et al.* 1999). Thus, while the DAT

decreases in the METH abusers may not be severe enough to induce Parkinsonian symptoms, they are sufficient to affect function.

As reported in the preclinical studies, the DAT losses in the methamphetamine abusers could reflect either degeneration of the DA terminal or a decrease in the expression of the DAT and/or in the processes mediating DAT trafficking and internalization (Melikan *et al.* 1999). In this respect it is interesting to note that the human postmortem study that documented reductions in DAT did not show changes in vesicular monoamine transporters 2 (VMAT2) in the METH abusers (Wilson *et al.* 1996). Because, VMAT2 are more stable markers of the DA terminals than DAT (Kilbourn *et al.* 1996), this was interpreted as reflecting persistence of the DA terminal. Further studies are required to determine if in humans the DAT losses reflect damage to the terminal or adaptation changes that may recover with detoxification.

Thus the imaging studies in human subjects have provided convincing evidence that there is a loss of DAT in METH abusers. However, the permanence of this effect and its significance in DA neurotransmission is unclear. This is particularly relevant for though it was initially believed that METH-induced DAT losses reflected neurotoxicity, recent studies showing recovery (Harvey *et al.* 2000; Melega *et al.* 1997) suggest that these may reflect adaptive changes that recover with detoxification.

### **Cingulate, insular and cerebellar activation during stimulant intoxication and craving**

Imaging studies by Howell and colleagues have shown that primates during cocaine self-administration, but not during passive administration, have marked activation of the anterior cingulate and insular cortices and of the cerebellum. Imaging studies in human subjects have also shown consistent activation of the anterior cingulate cortex after administration of stimulant drugs or after stimulation with cocaine-related cues that induced craving. In cocaine abusers, but not in controls, intravenous methylphenidate (a drug that, like cocaine, increases extracellular DA by blocking the DAT) increased metabolic activity in the anterior cingulate cortex (Volkow *et al.* 1999a). Since the anterior cingulate cortex is involved with attention and motivation (Devinshy *et al.* 1995<sup>xiv</sup>), its activation in the cocaine abusers, but not in the controls, was interpreted as reflecting the enhanced salience that methylphenidate had, in the cocaine abusers who report its effects, to be similar to those of cocaine (Wang *et al.* 1997). Activation of the anterior cingulate cortex in cocaine abusers has also been reported after administration of cocaine (Breiter *et al.* 1997<sup>xv</sup>) and during cue-induced cocaine craving (Maas *et al.* 1998<sup>xvi</sup>). Moreover, in one study in cocaine abusers, the degree of activation of the anterior cingulate cortex was associated with the intensity of the craving induced by a videotape purposefully designed to induce cocaine craving (Childress *et al.* 1999<sup>xvii</sup>). Activation of the anterior cingulate cortex is unlikely to be specific to stimulant intoxication or craving since it is also activated during cannabinoid intoxication in marijuana abusers but not in controls (Mathew *et al.* 1997<sup>xviii</sup>).

The insular cortex has also been shown to be activated in cocaine abusers during an interactive interview about cocaine themes designed to elicit cocaine craving (Wang *et al.* 1999). Moreover, in this study the activation of the insular cortex was significantly correlated with the self-reports of cocaine craving and with the increases in heart rate. The insula has multiple connections with limbic brain regions including the orbitofrontal cortex, amygdala, cingulate and hippocampus (Augustine *et al.* 1996; Chikama *et al.* 1997; Ghaem *et al.* 1997; Morecraft and Van Hoesen 1998) and with brain stem autonomic centers (Williamson *et al.* 1997; Phillips *et al.* 1997), so its activation could serve to integrate the emotional and autonomic responses observed during cocaine craving.

In humans, methylphenidate also produced marked cerebellar activation, but this effect was present both in cocaine abusers and in controls (Volkow *et al.* 1999a, 1997a). Moreover, the cerebellum was the brain region where methylphenidate induced the largest metabolic changes. Cerebellar activation has been reported to occur not only with other psychostimulants (Ernst *et al.* 1996) but also during marijuana intoxication (Volkow *et al.* 1996) and has been associated with craving-induced by cocaine-induced cues (Grant *et al.* 1996). Though the cerebellum is mainly associated with motor effects, there is evidence that it may participate in reinforcing properties of natural as well as pharmacological stimuli (Plotnik *et al.* 1992; Ball *et al.* 1974). The cerebellum has connections with limbic brain regions (Heath and Harper 1974), so that cerebellar activation could, via its neuroanatomic connections, activate regions directly involved with reward.

## IMAGING STUDIES ON COCAINE-INDUCED DOPAMINE TRANSPORTER BLOCKADE IN HUMAN SUBJECTS

PET studies have measured the levels of DAT blockade induced by cocaine doses that are perceived as reinforcing in cocaine abusers (Volkow *et al.* 1997b). These studies showed that intravenous cocaine at doses commonly abused by humans (0.3 to 0.6 mg/kg) blocked between 60 to 77% of DAT sites in these subjects. Moreover, the magnitude of the self-reported "high", which is a measure of the reinforcing effects of drugs in humans, was correlated with the degree of DAT occupancy. These studies also showed that at least 47% of the DAT had to be blocked for subjects to perceive cocaine's effects. Furthermore, the time course for the "high" paralleled that of cocaine's concentration in striatum, which is the brain region where the nucleus accumbens is located. The findings in human subjects are consistent with the results from those in non-human primates reported by Howell showing that reinforcing doses of cocaine led to greater than 50% blockade of DAT. In fact the ED50's (doses required to produce 50% occupancy of DAT by cocaine) obtained in non-human primates (0.1mg/kg-0.27 mg/kg) are very similar to those obtained in human subjects (0.25 mg/kg) (Fowler *et al.* 1998; Volkow *et al.* 1997b; Gatley *et al.* 1999).

### DA D<sub>2</sub> receptors modulate responses to psychostimulant drugs in humans

One of the most challenging problems in the neurobiology of drug addiction is to understand why some individuals abuse drugs while others do not. Studies in laboratory animals have provided evidence that DA modulates predisposition to drug abuse (Piazza *et al.* 1991) and that stressors facilitate drug self-administration (Piazza and Le Moal 1998). Nader and colleagues have shown for the first time, that a social stressor can modify DA D<sub>2</sub> receptor levels in non-human primates and that these changes predict the vulnerability to self-administration of cocaine. There are no equivalent studies in human subjects. However, imaging studies in drug abusers have consistently shown reductions in D<sub>2</sub> receptor availability in drug abusers (reviewed by Volkow *et al.* 1999b). Unfortunately, such studies cannot rule out the extent to which these changes reflect the chronic exposure of the drug, genetic differences and or environmental influences that may have predisposed subjects to self-administer drugs. To address the role that the differences in D<sub>2</sub> receptors may have on responses to stimulant drugs, PET studies have been done to assess if brain D<sub>2</sub> receptor levels predict the reinforcing responses to stimulants in humans (Volkow *et al.* 1997). For this purpose, studies measured the baseline levels of D<sub>2</sub> receptors in striatum of healthy non-drug abusing subjects and in parallel assessed the behavioral responses to the stimulant drug methylphenidate (a drug for which the reinforcing effects have been linked to blockade of DAT; Ritz *et al.* 1987).

Approximately half of the subjects described the effects of methylphenidate as pleasant and half as unpleasant. There were no differences in subjects' demographics, smoking status or in plasma methylphenidate concentration between individuals who liked and those that disliked methylphenidate. Subjects that described methylphenidate as pleasant had significantly lower levels of D<sub>2</sub> receptors ( $2.7 \pm 0.32$ ) than the subjects who described it as unpleasant ( $3.1 \pm 0.26$ ) ( $p < 0.004$ ). Moreover, D<sub>2</sub> receptor levels correlated negatively with methylphenidate-induced pleasant effects ("happy"  $r = -0.58$ ,  $p < 0.005$ ; "mood"  $r = -0.52$ ,  $p < 0.01$ ) and positively with its unpleasant effects ("annoyed"  $r = 0.53$ ,  $p < 0.01$ ; "distrustful"  $r = 0.66$ ,  $p < 0.001$ ).

The differences in response to methylphenidate between subjects with high and low D<sub>2</sub> receptors could be explained if there is an optimal range for D<sub>2</sub> receptor stimulation to be perceived as reinforcing; too little may not be sufficient but too much may be aversive. Thus, it is possible that in subjects with high D<sub>2</sub> receptors a smaller dose of methylphenidate may have been perceived as pleasant. If D<sub>2</sub> levels also modulate sensitivity to physiological reinforcers, then one could postulate that low D<sub>2</sub> receptors would predispose a subject to use drugs as a means to compensate for the decreased activation of reward circuits ("reward deficiency syndrome"; Blum *et al.* 1996). Alternatively it is possible that low D<sub>2</sub> receptors could predispose to psychostimulant abuse by favoring initial "pleasant" drug responses, which have been shown to predict future drug use (Davidson *et al.* 1993), and/or that high D<sub>2</sub> receptors may protect against drug abuse by favoring "unpleasant" drug responses.

This is the first evidence in humans linking D<sub>2</sub> receptors to the reinforcing responses to stimulants. These findings coupled with the results in non-human primates by Nader and colleagues provide evidence that differences in D<sub>2</sub> receptor availability modulate the propensity for drug self-administration. Thus, effects of social stress on D<sub>2</sub> receptors may be one of the mechanisms accounting for the differences in addiction rates as a function of social class (Wohlfarth and van den Brink 1998).

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## SYMPOSIUM VIII

### MEASURES OF IMPULSIVE BEHAVIOR IN THE CONTEXT OF DRUG ABUSE

*H. de Wit and J.B. Richards, Chairpersons*

Although we have a common understanding of the meaning of "impulsivity" from ordinary language usage, closer examination of the term suggests that it may refer to a range of behaviors, perhaps reflecting more than one underlying behavioral process. Researchers have proposed several operational definitions of impulsivity, and have developed standardized procedures to measure these. However, it is not known whether the different procedures and behavioral measures assess a single process, or different processes. Impulsive behavior plays an important role in substance abuse, both as a risk factor for using drugs and as a direct pharmacological effect of drugs. As a risk factor, it is thought that individuals who are high on the trait of impulsivity may be more likely to experiment with drugs, more likely to continue using drugs after initial experimentation, and have more difficulty abstaining from use once they have used the drug on a regular basis. Drugs may also direct pharmacological effect on behavior, making it more likely that users will exhibit maladaptive, impulsive patterns of behavior. Therefore, drug abuse researchers can benefit from research to clarify the structure and mechanisms underlying impulsive behavior.

Several operational definitions of impulsivity have been proposed, including a) a cognitive impairment, or an impaired ability to evaluate delayed or probabilistic outcomes, b) a motivational abnormality, relating to the integration of rewards, punishments, and probabilities, and c) a problem with inhibitory control. Each of these processes has some face validity, and each has been validated with certain behaviors that may be related to drug abuse. For example, several studies have used delay discounting procedures to show that drug users are less responsive to delayed, compared to immediate, rewards (see below). In other studies, it has been demonstrated that individuals with poor inhibitory control perform poorly on a reaction time task that requires rapid inhibition of behavior, and performance on this task is impaired by drugs of abuse, including alcohol. Other researchers have suggested that impulsivity is related to an enhanced sensitivity to reward or relatively low sensitivity to punishment. Finally, impulsivity has also been related to risk-taking and aggression. An important focus of current research is to determine how these various measures of impulsive behavior are related, and whether they reflect the same or different processes. Research is also focussing on how these behavioral tendencies relate to drug abuse, in terms of risk of initiation, continuation, difficulty stopping, and impairment while under the influence. Further studies are needed to apply our understanding of impulsive behavior to develop effective strategies for prevention and treatment, and to identify the neural mechanisms underlying the behaviors. The research summarized below addresses some of these important issues.

#### **TRYPTOPHAN DEPLETION IMPAIRS MECHANISMS OF AFFECTIVE LEARNING AND DECISION-MAKING BEHAVIOUR IN HEALTHY HUMAN VOLUNTEERS: IMPLICATIONS FOR UNDERSTANDING IMPULSIVITY**

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Heightened impulsivity is a feature of several psychiatric illnesses. However, the cognitive and neural basis of impulsivity is still largely unknown. In this paper, I presented recent results, obtained with computerized neuropsychological tests, suggesting that individuals with acknowledged impulse control problems — patients diagnosed with DSM-III-R borderline personality disorder (without current major affective disorder) — exhibit a complex of neurocognitive impairments that might help mediate their impulsive actions. These include difficulties in resolving between competing courses of action, and deficient inhibitory control in situations involving the modulation of a strongly activated or prepotent response. Both experimental and clinical evidence suggests that serotonin function may be important for both effective choices and behavioral inhibition. In the case of the former, reduced central serotonergic function in healthy human volunteers — achieved by dietary tryptophan depletion — has been shown to alter choices between actions associated with uncertain amounts of reinforcement and/or punishment (Rogers *et al.*, 1999). This suggests that serotonin modulates the functions of the orbital prefrontal

cortex. In this context, I shall present new, preliminary evidence that tryptophan depletion impairs human decision-making, in part, by altering the processing of reward-based information. These results may have implications for understanding how serotonin modulates choices between reward and resultant changes in mood in vulnerable individuals.

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## TWO MODELS OF IMPULSIVE BEHAVIOR USED IN RATS AND HUMANS

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Richards and de Wit have studied impulsive behavior using two paradigms in parallel studies in rats and humans. The first paradigm involves an impairment in inhibitory control of behavior and the other emphasizes a relative insensitivity to delayed consequences. Impaired inhibitory control has been described by some investigators as an inability to inhibit responses to reward related-stimuli, or by others as a stress-induced break down of control over previously inhibited behaviors (Shaham *et al.* 2000). In the second paradigm, impulsive behavior is seen as an impairment in behavioral choice and decision making, thought to result from a relative low value associated with delayed consequences (Ainslie 1975; Logue 1988; Rachlin 1995). Greater impulsivity is indicated by more rapid "discounting" of the value of delayed consequences.

Richards and de Wit have applied and developed procedures based on these two operational definitions in both humans and rats. The stop task procedure, initially developed by Logan (1994) for use with humans, measures inhibition as the ability to stop an ongoing prepotent response. Children with Attention Deficit Hyperactivity Disorder are impaired on this task, and their performance on the task improves when they are treated with methylphenidate, a drug that also improves their symptoms (Tannock *et al.* 1989). Richards *et al.* (1997) adapted the procedure for use with rats. The second task, delay discounting, provides a measure of the value of delayed and uncertain consequences, using an adjusting amount (AdjAmt) procedure, in which subjects choose between a series of delayed or immediate rewards. Other investigators have shown that drug dependent individuals discount the value of delayed rewards more rapidly than control subjects (Allen *et al.* 1998; Bickel *et al.* 1999; Crean *et al.* 2000; Kirby *et al.* 1999; Madden *et al.* 1997; Mitchell 1999; Vuchinich *et al.* 1998).

Richards and his colleagues have explored the direct effects of drugs on discounting in rats. In rats trained on the delay discounting procedure, acute moderate doses of amphetamine, which increases synaptic dopamine levels, decreased delay discounting (i.e., made the animals *less* impulsive), whereas D1/D2 type dopamine antagonists such as flupenthixol or raclopride had the opposite effect (i.e., made the animals *more* impulsive; Wade *et al.* 2000). They also tested the effects of repeated doses of methamphetamine, and found that 14 days of treatment with a relatively high dose of methamphetamine increased impulsivity on the delay discounting task (Richards *et al.* 1999). Thus, methamphetamine had opposite effects depending on whether it was administered acutely or chronically. Microdialysis studies have shown that baseline levels DA overflow are decreased after chronic treatment regimens of methamphetamine (Imperato *et al.* 1996), suggesting that the increase impulsivity after chronic methamphetamine administration may be due to diminished DA transmission.

de Wit and Richards have tested d-amphetamine, alcohol, and marijuana on the delay discounting procedure in humans, and found that none of these drugs affect delay discounting, even at doses that have robust effects on subjective report measures. The differences between the results of rat and human studies with delay discounting



may be due to procedural differences. For example, in the rat procedure, the animals experience both the rewards and the delays during each test session. In the human procedure, the subjects are asked their preferences for different rewards associated with various delays, but do not actually receive them during the test session. The direct experience of delays and rewards may be critical to detect drug effects on impulsive decision-making. Nevertheless, the delay discounting procedure with humans is sensitive to trait measures and personality factors. For example, psychiatric outpatients who exhibited frequent impulsive behaviors discounted the value of delayed rewards more than a comparison group of patients who did not exhibit impulsive symptoms (Crean *et al.* 2000). Furthermore, as noted above, several studies have now shown that drug dependent individuals exhibit stronger delay discounting than non-dependent individuals. Taken together, the results from the human studies indicate that the current discounting tasks may not be sensitive to the acute effects of drugs, and modifications may be needed to allow the subjects to experience the delays and rewards during testing. Nevertheless, the current delay-discounting task is sensitive to more lasting personality traits and the chronic use of drugs.

On the stop task, highly similar results have been obtained with rats and humans. For example, acute administration of amphetamine *improved* behavioral inhibition in subjects with initially poor inhibitory control, whereas it had no effect in subjects with good inhibitory ability. This effect was observed in both rats and humans. Rats and humans also perform similarly after acute doses of alcohol (de Wit *et al.* 2000; Feola *et al.* 2000). Alcohol impaired the ability to inhibit responses at doses that had no effect on simple reaction times. These results indicate that the stop task may prove to be a valuable transitional model between rats and humans.

Current studies by this research group are investigating the role of different neurotransmitter systems on the different measures of impulsive behavior. For example, it appears that the serotonin system is critically involved in the ability to inhibit responses, whereas the dopamine system is involved in the valuation of delayed consequences. The neurobiology underlying different forms of impulsive behavior, including behavioral inhibition and delay discounting is likely to be highly complex, and involve more than a single neurotransmitter system. Nevertheless, we can advance our understanding of these mechanisms by conducting systematic and carefully controlled behavioral studies with different models of impulsive behavior.

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## **IMPULSIVITY AS IMPAIRED INHIBITION: EFFECTS OF ETHANOL**

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Dr. Fillmore has studied the acute effects of moderate doses of alcohol on the ability to inhibit behavior in healthy adult social drinkers. These studies utilize procedures based on contemporary cognitive models of response inhibition, including the stop signal paradigm (Logan 1994), go no go tasks (Patterson and Newman 1993), and negative priming procedures (Tipper 1985). A number of theories postulate that behavior is governed by two distinct systems: one that activates behavior, and one that inhibits behavior (e.g., Fowles, 1987; Logan and Cowan, 1984). Much research has focused on processes that govern behavioral inhibition, implying that the impairment of inhibition underlies deficits of self control. Behavioral inhibition is generally defined as the act of withholding or terminating a behavioral response, and is considered to be governed by a cognitive inhibitory process (Logan, 1994).

Dr. Fillmore's presentation addressed the role of environmental context on impaired inhibition under alcohol, and how this behavioral disturbance might alter response strategies used in reward acquisition. His research used the stop signal task to measure inhibition. The task engages individuals in responding to go signals, and occasionally requires them to inhibit the response when a stop signal occurs. It has been suggested that depressant and anxiolytic drugs, such as alcohol, might temporarily weaken the behavioral inhibition system leaving the activation system to dominate behavior (e.g., Quay, 1997). Dr. Fillmore's research showed that a moderate dose of alcohol selectively reduces drinkers' ability to inhibit their behavior while leaving their ability to activate behavior unaffected (Mulvihill, *et al.*, 1997; Fillmore and Vogel Sprott, 1999, 2000). These findings support the notion that behavioral inhibition is particularly sensitive to the impairing effects of alcohol. Dr. Fillmore's presentation also addressed the role of environmental context on the display of impaired inhibitory control under alcohol. His data showed that impaired response inhibition under alcohol is not immutable but can be either intensified or reduced by several factors by rewarding monetary consequence made contingent upon inhibiting and activation-behavioral responses. When there was an imbalance between the consequences, by reinforcing either inhibitions or responses, inhibitory control was not affected by alcohol. Under those conditions, behavior was determined by the reinforcement. When inhibitions were reinforced, they increased, and when responses were reinforced, they became faster. The evidence challenges a purely pharmacological account of alcohol-induced disinhibition that assumes that the drug inevitably impairs the ability to inhibit or control behavior.

Dr. Fillmore suggested that acute alcohol induced impairment of inhibitory control could contribute to alcohol abuse. By highlighting evidence that even mild doses of alcohol can impair cognitive processes, Dr. Fillmore stated

that it is important to understand how such impairments might also affect control over drug intake. As a working hypothesis, he suggested that impairment of inhibitory processes during initial use of alcohol might subsequently compromise the ability of some people to stop repeated alcohol consumption. He presented the results of a study that tested the effect of alcohol on the inhibitory and activational response strategies used in the acquisition of alcohol and monetary reinforcers. A stop signal task tested the degree to which alcohol increased subjects' responding for the drug by biasing their reward acquisition strategies in favor of response activation, and away from inhibitory responding. Results showed that the preload dose of alcohol biased subjects' reward acquisition response strategies in favor of those dependent on activational processes, and away from those relying on inhibitory processes. Under alcohol, reward acquisition behavior was characterized by an increase in response speed that was accompanied by fewer successful inhibitions to stop signals. By contrast, those who received the placebo preload obtained rewards by meeting a combination of activational and inhibitory response criteria, each occurring with a similar likelihood.

Dr. Fillmore concluded by stating that alcohol impairment of inhibitory processes might represent a route through which an initial dose of the drug promotes continuing self-administration of the drug, potentially leading to binge drinking. He also mentioned the importance of better understanding the role of environmental conflict on impaired inhibitory control under alcohol, and how the drug might alter behavior in other conflict situations (e.g. temporal conflict).

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## DISCOUNTING: AN EVOLVED PSYCHOLOGICAL MECHANISM COMMANDEERED IN DRUG DEPENDENCE?

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Dr. Bickel and his colleagues have sought to understand the ultimate causes of drug abuse by placing drug dependence in an evolutionary context. Consistent with the notion that drugs of abuse commandeer brain mechanisms evolved to function with other reinforcers (e.g., Nestler and Landsman, 2001), these researchers suggest

that delay discounting is an evolved psychological mechanism that is commandeered and exaggerated in the drug dependent.

First, Bickel and his colleagues argued that delay discounting is an evolved psychological mechanism. Because of the uncertainty of resources in the environment of the wild animal or hunter/gatherer human, selective pressure was present for the immediate consumption of reinforcers such as food. Indeed, most animals spend more waking hours in the acquisition of food than any other waking activity, and evolutionary theorists have noted that diet is the chief issue influencing a species' other adaptations (Tooby and Devore 1987). Evidence to support the notion of delay discounting as an evolved mechanism is the qualitative similarity between discounting functions estimated in humans and other animals (e.g., Mazur 1987). Although humans have vastly lower rates of discounting, the qualitative shape of the functions appears to be fixed, suggesting common ancestry.

The second issue addressed was whether extreme temporal discounting is a general behavioral feature of the drug-dependent individual. This may explain why drug-dependent individuals often make decisions that do not adequately take into account the future consequences of their behavior. Evidence demonstrates that discounting of monetary rewards is greater in drug dependent individuals than matched controls, and that drug-dependent individuals discount drug rewards more than they discount monetary rewards. In early studies, these investigators showed that heroin addicts discount money more steeply than matched control subjects (Madden et al. 1997; see also, Kirby et al. 1999; Madden et al. 1999). For example, control subjects discounted a hypothetical thousand dollars by 50% in 5 years, whereas the heroin addicts discounted this amount by 50% in 1 year, a 5-fold difference (Madden et al. 1997). In another study, these investigators examined discounting of a hypothetical thousand dollars' worth of heroin in heroin addicts. In contrast to the previous study in which money lost 50% of its value in 1 year, heroin lost 50% of its value in 1 week. Similar results were obtained with cigarette smokers (Bickel et al. 1999). Smokers discounted money significantly more than non-smokers, and smokers discounted cigarettes more than money. Interestingly, ex-smokers, who were matched with the smokers and non-smokers on demographic characteristics, discounted monetary outcomes to an almost identical extent as non-smokers. This interesting result could be explained by two competing hypotheses. First, perhaps these ex-smokers discounted less than other smokers and that, in turn, made it easier for them to quit. Second, perhaps discounting is a reversible effect of drug use. Therefore, methods that reduce drug use should also reduce discounting.

Finally, to empirically test these competing hypotheses, discounting for cigarettes and money was assessed in moderate to heavy cigarette smokers who participated in a contingency management program to decrease their smoking. The participants were dependent smokers who smoked 20 or more cigarettes a day, but had no proximate plans to quit. The study had three phases: (1) baseline discounting determination, (2) random assignment to contingency management, or control conditions, and (3) post-intervention discounting determination. Subjects in the contingency management condition were instructed to stop smoking and they received money if their CO levels confirmed abstinence. Subjects in the control group were instructed to smoke as they normally would. After the intervention, discounting for cigarettes decreased in the contingency management group, but not in the control group. Notably, discounting for money also decreased in the contingency group, but remained unchanged in the control group. These data suggest that (1) abstinence leads to reduced discounting of both cigarettes and money, (2) discounting is related to current cigarette use, and (3) that the relatively high levels of discounting observed in smokers is a reversible phenomenon of drug ingestion.

Collectively, these studies suggest that delay discounting is an evolved psychological mechanism that may be commandeered in the process of drug dependence, resulting in exaggerated discounting in drug dependent individuals. Bickel and colleagues' recent results suggest that this increasing discounting is a reversible phenomenon. These results suggest that the discounting of future rewards may be an important process contributing to drug dependence.

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## **ALCOHOL-HEIGHTENED AGGRESSION AS IMPULSIVE BEHAVIOR IN LABORATORY RODENTS: ROLE OF SEROTONIN 1B RECEPTORS**

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The behavioral features of excessively aggressive laboratory animals are closely similar to those defining *poor impulse control* in humans (Coccaro, 1989), (Brown and Linnoila 1990)). Analytical tools have been developed that allow precise quantification of aggressive behavior in those individuals who are (1) readily *provoked* to engage in aggressive behavior (i.e., low thresholds, short latencies), (2) execute aggressive acts at *high rates* and in a non-ritualized *injurious* manner, (3) fail to terminate aggressive “bursts,” (4) not inhibited by *appeasement* signals, and (5) not influenced by adverse short- and long-term *consequences*. Operational definitions of “impulsivity” have emphasized preferences of immediate small payoffs over long-term gain ((Tobin and Logue 1994), (Evenden and Ryan 1996), (Monterosso and Ainslie 1999)). It remains to be determined whether highly impulsive aggressive individuals show a highly aggressive response to alcohol, stimulants or opiates, show high preference for these drugs, are unusually sensitive to the reinforcing effects of alcohol, stimulants or opiates, and take large amounts quickly without regard for ill consequences.

Experimental data with large samples of laboratory rodents reveal that (1) a significant subset consistently exhibits an unusual pattern of intense aggression, far in excess of the species-typical level, when under the influence of a moderate dose of alcohol (Miczek *et al.*, 1992), (Miczek *et al.*, 1993), (Miczek *et al.*, 1998). (2) The magnitude of this alcohol effect exceeds the statistical outlier criterion of 2 standard deviations of the individual’s average level of fighting under vehicle control conditions. (3) Alcohol-heightened aggression is present in outbred and several inbred lines of mice and in rats; it persists during repeated tests for several months; it is seen in socially and singly housed mice. These results point to a potential animal model for impulsive aggressive behavior.

However, when given access to alcohol via drinking bottles or as reinforcer for operant behavior, animals exhibiting excessive aggressive behavior under alcohol, do not show higher intake (Van Erp and Miczek 1997), (Miczek and de Almeida, 2001). Furthermore, animals exhibiting alcohol-heightened aggression perform not significantly different from alcohol-non-heightened aggressors, when challenged with tests during which reinforcement is omitted entirely (“extinction”) or available under progressively increasing response demands (“progressive ratio schedule”). In most studies the proportion of alcohol-heightened aggressive animals amounts to ca. 25-30% of the total sample, but this proportion can be doubled by exposing animals to repeated alcohol injections that sensitize their motor activity (Fish *et al.*, in press). This latter finding points to the induction of excessive levels of aggressive behavior after alcohol treatment rather than as a trait.

The role of serotonin in impulsive aggressive behavior under the influence of alcohol is based on two lines of evidence, one involving direct serotonin measurements and the other relying on anti-aggressive effects of serotonin 1B receptor agonists. Using *in vivo* microdialysis, serotonin in the medial prefrontal cortex undergoes no detectable changes during the initiation of an aggressive episode, but once in progress, cortical serotonin declines (Van Erp and Miczek, 2000). This result suggests a role of cortical serotonin in the termination of aggressive episodes. When serotonergic 1B receptors are activated, a specific inhibition of intense levels of aggression is achieved that is not accompanied by disturbances in motor activities (Fish *et al.* 1999), (de Almeida *et al.*, 2001), (Miczek *et al.*, 2001). The serotonin 1B receptor subtype is a particularly promising target for further development as pharmacotherapeutic target.

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## SYMPOSIUM X

### MULTIDISCIPLINARY OUTCOMES IN DRUG ABUSE, NETWORKS, HIV EVOLUTION, AND NEUROAIDS

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*K.A. Crandall<sup>6</sup>, and J.B. Page<sup>1,4,5</sup>, Presenters*

This article is composed of three parts with references combined.

#### PART 1. NETWORKS: PARADIGM, PARADOX, AND DILEMMA (JBP)

##### I. BACKGROUND OF THE NETWORK PARADIGM

**Why pursue a network paradigm?** In the earliest reports on AIDS, investigators suspected that close interpersonal contact had something to do with transmission of whatever pathogens had brought about the immune collapse of the first patients. William Darrow, the CDC's investigator of sexual contacts, traced the early epidemic from a "patient zero," based on his skill at eliciting the self-reported behavior of people who had sex with each other. Later, as the pathways between bloodstreams became more clearly defined, interpersonal connections appeared to hold the key to the behavioral control of HIV infection. If people could remember and report their sexual partners or the people with whom they used drugs, it seemed logical that the characterization of these connections would help investigators to understand how HIV moved from person to person and how to prevent that movement. As we shall see later, the relative stability of the virus and its long latency period presented problems in interpreting the interpersonal connections that molecular, social, and behavioral scientists were poised to characterize. Nevertheless, the behaviors associated with HIV infection, with the exception of transfusion, all involved private interaction, sexual or drug using behavior. Networks of informal social relations had long been an heuristic tool for the study of drug use and sexual behavior. Therefore, despite the difficulties presented by the nature of HIV, network paradigms received attention in the behavioral literature on AIDS.

**How network paradigms grew out of standard anthropological inquiry.** Anthropological research was relatively slow to focus on city-dwelling people, possibly because the earliest anthropologists were most interested in discovering ways of life radically different from their own. They fanned out across the world to collect data on ways of life from the Sudan to Siberia, from North America to Australia. They studied small villages, tribes, and chiefdoms, places where they could apply their holistic perspective and develop a sense of the human condition in all of its different aspects. Cities present challenges to this approach, because they routinely combine people of different cultural backgrounds. Also the anthropological staples for analyzing social structure – kinship, age grades, clans, and chieftaincies, are more difficult to identify and trace in cities than in small communities. Once anthropologists began to turn their attention to cities, they found that connections among people could be studied, but not in traditional anthropological ways. By the 1950s, the discipline was thirsty for a paradigm that would help them to study complex societies using anthropological methods.

##### A. First work

###### 1. Bott's (1957) characterization of working class social relations

In order to systematize the study of anthropological questions in cities, Elizabeth Bott (1956) devised a strategy of defining the **informal network of social relations**, which seemed to obtain where kinship and political structures seemed not to apply. In the London neighborhood where Bott studied working class people, social relations that included elements of kinship, personal preference, work-related association, like interests, and other varieties of connections all played parts in the establishment of links among people. She called the summary of these relations a network, and a very influential paradigm was born.

###### 2. Mitchell's African Examples

J. Clyde Mitchell (1969), an Africanist, encountered radically modified social structures among people of diverse cultural background who lived in the rapidly growing cities of Africa. To some extent, the inhabitants of these cities observed their customary social ties with family members and members of social structures belonging to their prior cultural backgrounds, but in their new, highly interdependent urban environments, they formed social ties of varying

strength with people outside of their cultural traditions. Mitchell characterized this trend, and developed a theory of network structure.

**a. Morphology**

Morphology involves the structure of the network, whether it is amorphous or centralized, and which linkages connect which actors. Mitchell identified two basic types of morphology, centralized and amorphous. In amorphous networks, connections between individuals appear to link together dyads in no particular pattern, with no figures that have more than two or three links to other participants in the network. Centralized networks have structures in which certain people have extensive connections with other participants, causing links to cluster around them in representations of the network.

**b. Linkage**

Linkage describes the reasons that people have for establishing social relationships. Many varieties of network analyses work only with one or two types of linkages (e.g. "speaks to"), but in fact, people have many reasons for associating with each other, including shared religion, school background, work details, drinking buddies, and so on. Computational characterizations of linkages generally are at a loss to reflect any complexity of linkage.

**3. Wolfe's typology of content elements**

Alvin Wolfe (1970) devised a scheme for describing the various kinds of linkages, so that information on networks in all of their variations could be characterized and compared. This scheme includes eight major headings, such as religion, workplace, recreational, etc. Although this scheme includes complexity of linkage, it has not produced any compelling paradigms.

**B. A Developing Paradigm**

**1. Killworth's and Bernard's CATIJ** – Using very basic criteria for characterizing linkage (i.e. "speaks to") and abstract mathematics, Killworth and Bernard (1974, 1979) characterized morphology of networks in closed systems (ships at sea, women's prison). Their formulae calculated social distance among actors in closed systems, demonstrating both the high complexity of problems in mapping networks and the inability of mapping paradigms to reflect complexity of content in linkages between actors.

**a. Content limitations** – Why people establish network links can be a highly complex question, as links usually involve multiple, interactive reasons.

**b. Strength of association** – How strongly links operate as part of human behavior patterns in complex social contexts depends partly on the content (providing motivation) and partly on personal preferences.

**1. AIDS**– Network analyses (Klovdahl 1985; Klovdahl *et al.* 1995) using more sophisticated software than that available to Killworth and Bernard attempted to extend the study of networks into a seemingly logical application, the study of HIV infection. The most well known application of this paradigm involved a study of gang members who had become HIV positive (Klovdahl *et al.* 1995) although it was clear that membership in the gang was related to HIV infection, this paper did not eliminate the possibility that factors outside gang activities and contacts contributed to the seroconversions in the sample.

**2. HIV and drug use** - Using a balance between morphology and content, Trotter (1995), Neaigus *et al.* (1995), and Woodhouse *et al.* (1995) attempted to apply network concepts to HIV spread among injecting drug users (IDUs). The problem with these approaches lies in their speculative conclusions. Rather than demonstrating convincing predictive value, they identify uses of network characterizations as possible tools in prevention. Trotter's network approach shows most utility in the Miami context (JB Page and P Shapshak, *in progress*, 2001).

**II. PROBLEMS WITH THE PARADIGM ITSELF**

**A. The diachronic dilemma** - Regardless of how thoroughly the investigator characterizes content of linkages and structure of a network's morphology, the resulting characterization, upon nearly instantaneous re-study of the same population, will be no longer true twenty minutes after the first characterization is complete. Over time, network links and structures constantly shift, and no scientific strategy for analyzing networks takes this fact into account, regardless of their cognizance of the problem.

**B. The HIV dilemma** - Because HIV remains latent for long periods of time, attribution of specific infection to specific exposure (and by extension, network linkage) presents difficulties. First, infected people often have histories of exposure to multiple others in the course of incurring risk of infection. Second, the virus itself does not reliably infect on all occasions of exposure. In fact, frequency of exposure plays a strong part in predicting eventual infection by HIV (McCoy *et al.* 1994, 1998).



C. **The content dilemma** – If a link between people has only one dimension, or strand, it will not last. If a link has multiple, strong strands, it may persist over decades. In the case of research among IDUs, linkage may vary in its complexity, but the participants are recruited because of their affinity for one strand – the use of illegal drugs. Therefore, the links upon which the researchers base their recruitment are likely to shift constantly. In the tracing of HIV infection among IDUs, models of frequency appear stronger than models of specific contaminated links (see Allard 1990). Another reason for this discrepancy lies in the medium of contamination. Among IDUs, needles, syringes, cookers, cottons, and standing rinse water all offer opportunities for contamination and exposure (Shapshak *et al* 2000a). What happens to these materials after one usage varies greatly, but it often involves impersonal transfer to a second user who may not be acquainted with the first (cf. Page, Chitwood *et al.* 1990). Running partners may never use the same needle, because they each get one from the get-off's (i.e. shooting gallery's) coffee can before shooting up. Whoever contaminated the needle last is the source of infection for the current user, not the long-time partner. Therefore, unless the researcher can characterize the injection and sexual behavior of the individuals participating in a given network, the pathways of infection will be very difficult to trace.

### III. PRESENT RESEARCH

We are currently engaged in studies that may help to disentangle the conceptual confusion about the role of interpersonal networks in HIV infection. In a first attempt to use virus phenotype to trace pathways of infection, we addressed the dilemmas as follows:

A. **Addressing the diachronic dilemma** – Having followed some IDUs for as long as 10 years, we identified certain dyads among them as having long histories of close contact and restricted exposure through risk behavior, including use of contaminated needles and unprotected sex.

B. **Addressing the HIV dilemma** – Because the selected dyads had histories of very limited contact with risk outside of the dyad, we could assume repeated contaminated exposure between the persons in the dyad.

C. **The content dilemma** – We had so thoroughly characterized the linkage between partners in the dyad that we could be reasonably certain of the dyad's permanence, and the stability of its strands. It was, therefore, not necessary to depict the additional strands in each relationship. All were multiple, strong, and of long duration, including the sexual and drug using strands.

### IV. FINDINGS BASED ON DYADS

A. **The laboratory analyses** (using phylogenetic and signature assessments) focused on four different aspects (Shapshak *et al.* 2001b) of the HIV found in the study participants: 1) V1-V5 env sequence data, 2) cell tropism, 3) N-linked glycosylation sites, and 4) CD-4 binding site.

B. **Congruency with dyads** – Variation of HIV strains between participants in the same dyad in two cases indicated strong inferred relationship between strains found in component individuals. Participants 1001 and 1002 demonstrated these relationships in all of the criteria mentioned above.

C. **Anomalies in dyads** – Of the other four dyads studied, we found highly disparate strains with one exception, an across-dyad relationship not predicted by the information available on the content of links among the people being studied. Participant 1005 showed few dissimilarities in the parameters studied when compared with 1001 and 1002. No direct evidence of intimate linkage between 1005 and the dyad consisting of 1001 and 1002 had emerged from the study team's previous investigation of the social contexts in which these individuals operated. Nevertheless, historical connections may account for the similarities in HIV strain among these three people. Participant 1004 showed no similarities to other participants' strains of HIV, but her internal variability provided reassurance of the integrity of the laboratory procedures. The latest HIV sequence findings reviewed below indicate new tightly associated sequences in drug abuse exhibiting restricted heterogeneity.

D. **Interpretation** – Even with the best possible ethnographic, longitudinal perspective on people at risk for HIV infection, and the most sophisticated possible strategies for characterizing strains of virus, the ability to trace infection among IDUs is far from airtight. Nevertheless, we have succeeded in identifying some of the parameters that may be useful in tracing HIV transmission, and this success points out where we need to direct our energies in order to advance the field in the question of tracing infection among networks of IDUs.

## PART 2. METHODS FOR THE ANALYSIS OF HIV EVOLUTION (KAC)

### I. EVOLUTION OF HIV: RECOMBINATION AND PHYLOGENETIC RECONSTRUCTION

A phylogeny is a set of relationships among groups of genes or organisms that reflects their evolutionary history. Inferring a phylogeny is an estimation procedure, a statistical inference of a true phylogenetic tree that is unknown. However, the goal of the phylogenetic analysis is not merely the reconstruction of a tree topology; the phylogeny provides a powerful framework in which several hypotheses can be tested and parameters of interest can be estimated from the data (Huelsenbeck & Crandall 1997). Once a reliable estimate of the phylogeny of the sequences under study has been obtained, it can be used to test diverse hypotheses about evolution. All phylogenetic methods are based on some set of assumptions. To understand the scope of the derived inferences, the assumptions of a method must be explained and delimited, and then tested and contrasted with the biological data at hand. Inferences derived from the phylogeny can be only as good as the phylogenetic estimate from which they were derived.

There are many reasons why a clear understanding of the genetic relationships among different strains of a virus is desirable. Such knowledge can provide information on the origins and geographic distributions of particular strains, on their routes of transmission, and for the development of vaccines (Crandall et al. 1999). In the case of HIV, its rapid evolution provides an ideal system for a successful application of a variety of phylogenetic approaches, as evidenced by the increasing number of studies on HIV using phylogenies. Phylogenetic analyses of HIV sequences have been used to investigate a variety of problems (Crandall 1999; Rodrigo & Learn 2001). These problems include potential transmission of the HIV virus among individuals (Ou et al. 1992; Hillis & Huelsenbeck 1994; Crandall 1995), cross-species transmissions (Sharp et al. 1995; Sharp et al. 1996), origins (Gao et al. 1999), Epidemiology (Holmes & Garnett 1994; Holmes et al. 1995; Holmes et al. 1999), subtyping (Voevodin et al. 1996) and drug resistance (Crandall et al. 1999). Phylogenetic studies have been critical for understanding the biology and evolution of HIV (Hillis 1999). In fact, the wealth of data accumulated over the last few years has made the immunodeficiency viruses the most data-rich group of organisms for any evolutionary analyses. In this chapter, we discuss procedures for estimating phylogenies from DNA and protein sequences, including hypothesis testing and applications, and point out diverse special concerns about HIV. This section gives some simple guidelines for the phylogenetic analysis of HIV sequences, including references to more specific reviews and available software. Swofford *et al.* (1996) provide the most comprehensive current review of the phylogenetic methodology, whereas Hillis (1999) and Posada *et al.* (2001) provide recent reviews of applying phylogenetics to HIV sequence data.

### II. PHYLOGENETIC RECONSTRUCTION

Phylogeny reconstruction is a complex process that requires several steps. Each step is equally important and should be completed carefully. In the next section, we outline the main phases in phylogenetic analysis: alignment, selection of optimality criteria and search strategies for optimal trees, use of appropriate models of evolution, and confidence assessment.

**A. Alignment.** The first step in any evolutionary study is to establish homology. In DNA sequence analysis, one hypothesizes that the nucleotides observed at a given position came from the same position in the common ancestor of the taxa under study (Swofford *et al.* 1996). This statement of positional homology constitutes an alignment. In the alignment, positions inferred to be homologous are in the same column of the data matrix, so insertions or deletions (indels) are postulated by inserting gaps in one or several sequences to maintain an "optimal alignment". The quality of an alignment is measured as some cost resulting from different penalties. The insertion of a gap, its size, or position can each be penalized in different ways. In general, penalties are bigger for gaps than for mismatches, as indels are usually rarer than substitutions. The cost is also bigger for internal gaps than for leading or trailing gaps, as the latter usually represent different lengths of sequences rather than actual evolutionary changes (Swofford *et al.* 1996). Also, a matrix of change costs may be specified for the different nucleotide substitutions, thereby allowing, for example, the specification of different costs for transitions and transversions. In the case of protein-coding sequences, information about the protein reading frame or about the secondary structure of the protein can be incorporated in the alignment process (see Kjer 1995). For example, gaps that are not multiples of three can be penalized more heavily than those that are multiples of three, because the former produce a shift in the protein (amino acid codon) reading frame.

Although alignment methods can be efficient, especially when the sequences are similar, they are not foolproof, and manual refinement of the alignment may still be required. Regions of the sequence alignment with substantial numbers of gaps, in which positional homology is too uncertain, should be omitted from the analysis (Swofford *et al.* 1996). However, deleting all the gapped columns—a procedure known as “gap-stripping”—results in an unnecessary loss of information that neglects the reality of indels as evolutionary events. Some phylogeny methods (e.g., maximum parsimony) can treat gaps as a fifth state, acknowledging the evolutionary reality of indel evolution. Some models of indel evolution (Thorne *et al.* 1992; McGuire *et al.* 2001) have been proposed for use in likelihood or distance analyses, but no widely available software programs currently implement these models. Therefore, indels are often treated as missing data in maximum likelihood and distance analyses, thereby resulting in some loss of information.

**B. Optimality Criteria and Searching Methods.** Once an alignment has been proposed, several methods can be used for estimating the phylogeny of the sequences under study. All commonly used phylogenetic methods have two parts: the specification of an optimality criterion, and the specification of a search strategy to find optimal or near-optimal trees. The optimality criterion is a statement of how goodness-of-fit between data and alternative hypotheses is measured, whereas the search strategy is the means for looking for the best tree among the universe of possibilities. Given an optimality criterion, a score can be assigned to each possible phylogenetic hypothesis, so that all the different hypotheses can be ranked in order of preference. The main optimality criteria used in phylogenetics are maximum parsimony, maximum likelihood, and minimum evolution. For any of these criteria, searches for optimal solutions can be quick and approximate (e.g., neighbor joining, stepwise addition) or thorough and exact (e.g., branch-and-bound, exhaustive searches). A comparative review of optimality criteria and search methods as applied to HIV analyses is given in Hillis (1999), so this discussion will not be repeated here. We will only note that each of the three optimality criteria has advantages, and that thoroughly searching for optimal solutions is often of much greater importance than which of the three optimality criteria is selected. Parsimony and minimum evolution analyses of DNA and protein sequences can be implemented in programs such as PAUP\*, PHYLIP, MEGA, and PHYLO\_WIN. Maximum likelihood methods for DNA sequences are implemented in PAUP\*, PHYLIP, PHYLO\_WIN, fastDNAML, PAML, MOLPHY, and GAML. Maximum likelihood analyses of protein sequences are implemented in PAML and MOLPHY. These programs and other phylogenetic software are summarized at Joe Felsenstein's website: <http://evolution.genetics.washington.edu/phylip/software.html>.

### III. MODELS OF EVOLUTION

Models of evolution are used in phylogenetic analyses to describe changes in character state, i.e., the rate of change from one nucleotide to another. The first model developed for molecular evolution was that of Jukes and Cantor (JC), who considered all possible changes among nucleotides to occur with equal rates. Other authors have suggested the incorporation of more realistic assumptions into these models. For example, base frequencies often differ among nucleotides and therefore may affect the rate of change from one nucleotide to another. Likewise, many genes show a bias in transitions over transversions, again affecting the rate of change from one nucleotide to another. We can incorporate these differences in rates of change by incorporating different rate parameters. Ultimately, for a symmetrical change model without considering codon position, we can have ten parameters, six rate parameters and four nucleotide frequency parameters. Of these ten parameters, eight can vary since the nucleotide frequencies must add to one and the rates are relative to a single change occurring with rate one. Given a large number of parameters to choose from, we wish to optimize a model for our particular data set.

It seems intuitive that a simple model like K80 may not adequately represent the complexity of the nucleotide substitution process in the HIV-1 virus (Muse 1999). One possible solution to model selection for constructing HIV-1 phylogenies could be the arbitrary use of complex (parameter-rich) models (e.g., Korber *et al.* 2000). However, this approach has several disadvantages. First, a large number of parameters need to be estimated, so the analyses become computationally difficult and require larger amounts of time. Second, the use of complex models increases the error with which each parameter is estimated. Ideally, we would like to incorporate as much complexity as needed in the estimation procedure. Indeed, this best-fit model of evolution can be chosen through rigorous statistical testing (Goldman 1993; Huelsenbeck & Crandall 1997; Posada & Crandall 1998; Posada & Crandall 2001). The relevance of model selection becomes apparent when the use of one model of evolution or another changes the results of the analysis (Kelsey *et al.* 1999).

#### IV. DETECTING RECOMBINATION

Phylogenetic inference can become questionable when the sequences under study have undergone recombination (Sanderson & Doyle 1992; Posada *et al.* 2001). Because there is ample evidence for recombination in HIV-1 sequences (Robertson *et al.* 1995; Robertson *et al.* 1995; Crandall & Templeton 1999), it is imperative that one test for recombination among sequences before subsequent studies are pursued. Not only will recombination influence phylogeny reconstruction, but also molecular clock hypotheses (Schierup & Hein 2000) and the estimation of population genetic parameters (Schierup & Hein 2000). However, there are many methods available for detecting recombination (reviewed in Crandall & Templeton 1999). How is one to choose the most appropriate method? Recent studies indicate that there is no single superior method, but that different methods perform better or worse depending on the level of genetic variation and the amount of recombination (Wiuf *et al.* 2001). In general, the detection of different patterns of substitution without relying on phylogenetic reconstruction perform best.

#### V. APPLICATION OF PHYLOGENETICS TO HIV STUDIES

Phylogenetic approaches have proven powerful in studying the evolution of HIV-1. For example, researchers have utilized phylogenies to explore the origin and spread of retroviruses such as HIV-1, HIV-2 and SIV through a population (Hirsch *et al.* 1989; Gojobori *et al.* 1990) and to identify transmission events among individuals and between species (Ou *et al.* 1992; Crandall 1995; Holmes *et al.* 1995; Sharp *et al.* 1996). Phylogenetic studies have also been used to examine the population dynamics of viral infections and the associations of host/pathogen (Harvey & Nee 1994; Holmes & Garnett 1994). Phylogenies have played a central role in longitudinal studies examining the diversification of HIV through time (Kuiken *et al.* 1993; Strunnikova *et al.* 1995) and how this nucleotide diversity is associated with compartmentalization within the body (Epstein *et al.* 1991; Ait-Khaled *et al.* 1995; Strunnikova *et al.* 1995; Shapshak *et al.* 1999) and related to gene function (McNearney *et al.* 1995). Finally, phylogenetic analyses have played a central role in the classification of HIV sequences (Robertson *et al.* 2000). Clearly, phylogenetic analysis has played a central role in the study of the evolution of HIV infection, transmission, and diversification.

### PART 3. DRUG ABUSE, NERVOUS SYSTEM, AND HIV STRAINS (PS)

#### I. INTRODUCTION

HIV exhibits extreme sequence heterogeneity, genetic variability, compared to most other terrestrial organisms. The HIV reverse transcriptase enzyme displays extreme recombinogenicity (the ability to jump among nucleic acid strands during replication) and lack of fidelity (incorporation of mis-matched [non-Watson-Crick] base pairs also during replication). There have been multiple introductions of HIV derived from SIV into humans: HIV-2 from the Sooty Mangabey and HIV-1 from the Chimpanzee (reviewed by Peeters and Sharp, 2000.) Today there are three main sub-groups of HIV-1. These three categories are 1. M 'main' comprising the assortment of known sub-groups (quasi-species or swarms) including A, B, C, D, F, G, etc; 2. N 'new' or non-M, non-O and 3. O 'outlier' found in Cameroon and West Africa (Peeters and Sharp, 2000.)

There are increased numbers of "CIRCULATING RECOMBINANT FORMS" [CRF] of HIV. The most surprising finding is that CRFs and other recent strains show high degrees of homology (~99%). Thus, it is surprising that Sub-group E has never been identified and those that were identified as E were actually CRFs of A/E sequences. In fact, there is very low diversity in new epidemiological strains currently being characterized. This may contradict earlier criteria for strain definitions (Korber *et al.* 1995; Learn *et al.* 1996). Currently, definition of a new sub-type involves the following three criteria: 1. They cannot be unique; 2. Three full-length genomes or two plus partial sequence of one must be known; and 3. They must be found in three individuals NOT Epidemiologically linked (Robertson *et al.* 2000).

The general principle appears to be that as more strains of HIV-1 overlap geographically they mix, new CRFs are produced, and thus the epidemic becomes more complex. However, the astonishing aspect is the extremely low heterogeneity of the new strains of HIV-1. Drug Abuse in these geographic regions appears to impact on strain production as discussed below.

## II. HIV-1 REPLICATION *IN VITRO* AND *IN VIVO* AND DRUG ABUSE (DA)

Abused drugs including cocaine, opiates, and morphine affect HIV-1 replication and HIV-1 disease. Opiates stimulate HIV-1 in PBMNC cultures that is reversed by naloxone. Cocaine also stimulates HIV-1 replication and may function through transforming growth factor- $\beta$  (TGF- $\beta$ ) [Henderson *et al.* 1991; Peterson *et al.* 1987, 1990, 1991, 1992.] Cocaine effects increases in HIV production in lymphocyte and neural cell cultures [Bagasra *et al.* 1993; Fiala *et al.* 1996; Shapshak *et al.* 1994, 1995]. Some effects of morphine may be via Fos/Jun signaling via AP-1 sites in the LTR. Morphine activates the HIV-1 LTR-CAT in human neuroblastoma cells. Greater effects were shown with PMA used to differentiate the cells [Squinto *et al.* 1990].

Are there biological correlates of the degree of diversity related to Drug Abuse? The LTR regulates virus transcription. Sites in the LTR include NF $\kappa$ B, AP1, and three SP1 sites. There are four different configurations of these sites among strains A, B, C, D, F, G, and CRF01\_AE. This supports the possible selection of strains due to Drug Abuse (McCutchan 2001). There are different rates of spread with different HIV subtypes. In Thailand, subtype B has similar rates for CRF01\_AE (Holm-Hansen *et al.* 2000.) In Kenya, subtype D is spreading faster than A (Neilson *et al.* 1999). In Sweden, rates are similar for subtype A, B, C, and D (Kaleebu *et al.* 2000). In Senegal, subtypes C, D, and G spread are 8 times greater than subtypes A and CRF01\_AG1bNG (Kanki *et al.* 1999). Are these differences due to earlier introduction or difference in virulence? It is interesting to note that one possible bridge between *in vitro* and *in vivo* studies appear counter-intuitive. On the one hand in culture (*in vitro*), abused drugs enhance heterogeneity of HIV-1 [Carneiro *et al.* 1999]. On the other hand, there is limited heterogeneity of sequences from current IDU studies worldwide.

## III. HIV STRAINS AND DA

Several questions are raised for the analysis of HIV strains and Drug Abuse. Are there hot spots of particular mutations in the HIV genome associated with risk activities of human hosts? If so then these could be areas targeted for therapy including strain specific vaccines. This is an important possibility because of the heterogeneity of HIV-1 and its sub-strains. Will the current indications of recombinant strains of HIV results in strain singularity and expansion ultimately or strain amalgamation? Are there biological correlates of degree of diversity related to Drug Abuse?

New recombinant HIV-1 strains became apparent since 1995 and were due to strand-switching activity of the viral reverse transcriptase enzyme. This occurs during co-infection of cells in co-infected individuals. Recombinants are clearly produced by interaction of viruses during co-infection. But what still remains to be elucidated are the mechanism of how the production of the strains occurs. All the new IDU transmissions and recombinant and non-recombinant strains appear to show restricted heterogeneity or greater homology ( 97%) (McCutchan, 2000). Subtype C is the primary worldwide strain (McCutchan, 2000). However, there is less recombination among sub-types A, C, and D (Becker-Pergola *et al.* 2000; Brodin *et al.* 1999).

Heroin IDU transmission is on the increase in South East Asia with several ingresses into China. (Beyrer *et al.* 2000). Strain CRF01\_AE appears spreading across North Vietnam and Guangxi province of Southern China. (Kato *et al.* 1999). Strains spread into Yunan and Guangxi provinces are distinct (Chen *et al.* 2000; Yu *et al.* 1999). Subtype B/C recombinant strains is spreading from Yunan to Xin Jiang province in Northern China (Shao *et al.* 2000). B/C recombinant strains are spreading more than CRF01\_AE in Guangxi (Yu *et al.* 2000). In Nepal low diversity C strain is spreading (Oelrichs *et al.* 2000). Although CRF01\_AE exploded in Thailand, there are more than 30 other unique CRFs. Thus, Injection Drug Use (IDU) activity has resulted in the introduction and spread of the A/E CRFs in Thailand and then into Northern Vietnam and Southern China through drug trafficking networks. Heterogeneity has not increased but increased spread has occurred due to recombination associated with co-infection. Among US military personnel, A/E is on the highest rate of increase although B currently still predominates. In China two different BC recombinants due to IDU have been detected. (McCutchan 2000). In an apparently related phenomenon, heroin-related IDU transmission is on the increase in Eastern Europe. Low diversity strains (A, B, and A/B) are increasing in Russia and the Ukraine (Bobkov *et al.* 1997, 1998) and similar phenomena were found in Belarus and Latvia (Ferdats *et al.* 1999; Lukashov *et al.* 1998; Novitsky *et al.* 1998).

#### IV. NEUROAIDS, BRAIN HIV STRAINS, AND DA

Models of CNS pathogenesis include disarray of expression of many genes in the host (P. Shapshak and C. Petito, in progress, 2001). These include for example, disequilibria networks of cytokines, chemokines, receptors involved in calcium ion transport, and enzymes involved in free radical production involving oxygen and nitric oxide species (reviewed by Shapshak *et al.* 2000b, 2001a). Strains of HIV-1 may also be involved in AIDS Neuropathogenesis. There are possibly genetic markers on the HIV-1 gp120 envelope C2/V3 region associated with the expression or absence of Cognitive Motor Complex in HIV infected patients. Geographical (in the USA and Europe) as well as molecular hot spots (in the HIV envelope C2/V3 regions) were identified and may be indicative of this phenomenon (Jurado *et al.* 1999). Thus it remains to be determined whether the cognitive motor related strains of HIV may be further identified over time. Other studies, including from our laboratory showed that there are specific strains of HIV-1 that infect different regions of brain (Chang *et al.* 1997; Cunningham *et al.* 1998; Shapshak *et al.* 1999.) In earlier studies others and our laboratory showed that there were differences among blood, CSF, and brain HIV-1 strains. (Ait-Khaled *et al.* 1995; Chiodi *et al.* 1988; Shapshak *et al.* 1995, 1996). Chen, Wood, and Petito (2000) showed that brain and spleen HIV sequences clustered separately whereas sequences from the Choroid plexus had both. Is the Choroid plexus thus a source or sink for HIV sequences into the brain or blood?

Drug abuse has deleterious effects on the brain. DUs had a higher level of HIV encephalitis, giant cells, and/or HIV p24 immuno-positivity than homosexual men with AIDS. Frontal lobe white matter productive infection, as shown by HIV p24 positivity, but was also present in grey matter in HIV encephalitis cases. HIV in grey matter was associated with cognitive impairment. HIV-1 proviral load was determined by quantitative PCR (separately in grey and white matter), and correlated with the presence of HIV encephalitis. No correlation was found between AZT therapy and the degree of cognitive impairment. Neocortical productive HIV infection is an important factor in dementia in AIDS or is this a reflection of higher viral burden in the brain (Bell *et al.* 1998).

Brain-derived HIV-1 V1-V2 envelope sequences from demented and non-demented AIDS patients displayed significant sequence differences and separate clustering between clinical groups. HIV-1 env diversity may influence the release of neurotoxic molecules from macrophages. This variation could account in part for the variability in the occurrence of AIDS dementia (Power *et al.* 1998). Variations in the possible use of host CCR5 and CCR2b co-receptors by specific HIV-1 V3 sequences was also implicated in differences in AIDS dementia (Smith *et al.* 2000). Brain-derived HIV-1 env sequences displayed greater evolutionary distance than B subtype brain-derived viruses for AIDS patients from Nairobi infected with HIV-1 A or D sub-types. Comparisons of both B and non-B subtype brain-derived viruses exhibited a preference for the CCR5 coreceptor (Zhang *et al.* 2001).

Another HIV dementia model has been developed as well with interesting implications that should also be explored further. The proposal is that trafficking of bone marrow-derived monocytes into the deep white matter during late stage of HIV-1 infection may be a critical step in the development of AIDS dementia (Liu *et al.* 2000).

Viral replication of HIV isolates from different lobes of brain from patients with and without AIDS dementia showed differences on various primary cell types (monocytes, monocyte-derived macrophages, and T cells). Replication patterns were different for HIV-1 strains from patients with and without AIDS dementia. This supports the idea of strains specific for AIDS dementia and neuropathology (Smit *et al.* 2001).

Trujillo *et al.* (1993) demonstrated immunological cross-reactivity of HIV-1 with brain proteins, thus increasing the potential mechanisms for brain damage due to HIV infection and the ensuing inflammation and immune response. This is also an area that could be explored further due to the influence of Drug Abuse.

#### V. CONCLUSIONS

Networks of informal social relations among people in highly complex urban environments offer intriguing paradigms for studying and explaining human behavior as it continues to evolve. In the case of HIV and its Epidemiology, networks present a tempting array of potential strategies for understanding how sexual partners or injection companions pass HIV from one to another. Nevertheless, we should be aware of the limitations of network paradigms in general, and especially regarding their application to questions of the spread of HIV. We have attempted to take into account these limitations in a study of HIV strain, finding that even the most thoroughly

characterized links between individuals do not necessarily predict the infection pathways found among IDUs based on nucleotide sequencing. We have begun to address the issue as to whether molecular genetic studies of HIV evolution may support, contrast with, and enlarge our understating of the HIV and drug abuse epidemics. The next order of complexity involves the possible prevalence of HIV strains associated with AIDS Dementia. At the molecular level information is obtained not necessarily predicted by the epidemiologist's approach. Thus molecular studies complement and enhance Epidemiological paradigms.

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## SYMPOSIUM XIII

### WHEN MARS MEETS VENUS: GENDER DIFFERENCES IN DRUG DEPENDENCE

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K. Brady and C.L. Wetherington*

#### INTRODUCTION

The study of gender differences in human behavior has always been of interest to researchers from a variety of areas of science. Although early research focused on basic anatomical differences between men and women, scientific inquiry soon spread to studies of personality and emotional well-being. Research found dramatic male-female differences, but it was only recently that such differences were supported by the discovery of anatomically different brain structures in men as compared to women. Research conducted by humorists, such as Dave Barry and Erma Bombeck, have found female brains to have large cerebral areas focused on shoe shopping as well as shoe and handbag coordination. In contrast, minimal space is devoted to spatial and directional abilities. For the male brain, large areas are devoted to sexually-related thoughts and activities as well as football, with much less cerebral space devoted to domestic skills and child rearing.

The purpose of this symposium was to examine gender differences in drug dependence. Specifically, Dr. Chilcoat reviewed epidemiologic data on gender differences in drug use, abuse and dependence. Drs. Miles and Pickens discussed the etiology of drug use, abuse and dependence, and the relative contributions of genetic and environmental factors to the development of these disorders. Dr. Lukas reviewed gender differences in human laboratory studies of drug abuse and dependence, followed by Dr. Kaltenbach's presentation on gender-specific treatment for drug use disorders. Gender differences in psychiatric comorbidity related to drug use and dependence was presented by Dr. Brady, with a discussion of future directions for research by Dr. Wetherington, from the National Institute on Drug Abuse.

#### EPIDEMIOLOGY

Current epidemiologic evidence of gender differences in various stages of drug use has been examined. This evidence ranges from initial opportunities to use drugs all the way to development and persistence of drug dependence. Findings from the National Household Survey on Drug Abuse (NHSDA) indicate that males are almost twice as likely as females to have used illicit drugs. Similarly, results from the National Comorbidity Study (NCS) indicate that men are generally twice as likely as women to have a history of drug dependence. Recent studies by Anthony and colleagues have shed light on the source of these gender differences. For example, Van Etten and Anthony (1999) found males were twice as likely as females to have been exposed to an opportunity to use marijuana or cocaine. Given equal opportunity for drug use, there are no longer any gender differences. Anthony *et al.* (1994) also found that gender differences in drug dependence were largely accounted for by differences in use – males were more likely to use various illicit drugs, but given use, both genders were equally likely to become dependent. One exception to this pattern was marijuana, for which dependence was twice as prevalent among male as compared to female users.

In contrast to illicit drugs (marijuana, cocaine, heroin), there were no sex differences for dependence on extramedical use of typically prescribed drugs, such as sedatives, anxiolytics and analgesics regardless of status. An additional analysis, which took history of conduct disorder, antisocial personality disorder and depression into account, tested the role of these factors as confounders. For dependence on prescription drugs, women were slightly more likely than men to be dependent given use, adjusting for conduct, and antisocial personality disorders. This finding was strongest for stimulant dependence, in which women reporting use were nearly twice as likely to develop dependence after adjusting for these disorders (Odds ratio = 1.94, 95% Confidence Interval = 1.25, 2.99). A final analysis explored whether there were gender differences in persistence of drug dependence. Although small sample sizes limited the ability to make statistical inferences, findings suggest that for illicit drugs such as marijuana, cocaine, and heroin, males are more likely to have dependence that persists compared to females. For typically prescribed drugs, persistence is similar for males and females.

These data indicate that gender differences in drug dependence are largely accounted for by gender differences in drug use. Interestingly, exposure to drug use opportunities is often overlooked in the progression to drug abuse, and it is this stage where gender differences emerge. Further understanding of why males are more likely than females to be exposed to opportunities to use drugs could provide important clues for preventing drug use and subsequent problems.

## ETIOLOGY

The role of gender in the etiology of adolescent drug use has been investigated. Previous studies have consistently found a significant genetic influence on the variance of drug use. Traditionally, studies have focused on male subjects and the few studies to include females have suggested that heritability estimates are somewhat lower in females.

The role of genes and environment on average monthly alcohol consumption and lifetime marijuana use among male and female adolescents (ages 13-21) who participated in the National Longitudinal Study of Adolescent Health, a school based study representative of the US general adolescent population, has been assessed. Data were available from 738 twin pairs (144 monozygotic male, 145 monozygotic female, 131 dizygotic male, 114 dizygotic female, 204 dizygotic opposite sex). Structural equation modeling using the program Mx revealed that the proportion of variance in adolescent marijuana use, that is due to additive genetic influences is similar for both males and females (29% and 24%, respectively). Thus, the overall influence from the environment was similar for the sexes. However, the magnitude of common environmental factors was much greater among adolescent females (58%) as compared to adolescent males (37%). Unique environmental factors were more important for male (34%) as compared to female marijuana use (18%). For alcohol use, additive genetic factors accounted for 16% to 23% of the variance for females and males, respectively. The magnitude of common environmental factors was again greater among adolescent females (52%) as compared with males (33%) and unique environmental factors were more important for male (44%) as compared with female alcohol use (32%). The genetic correlations between males and females for frequency of marijuana use ( $r = .06$ ) and alcohol use ( $r = .15$ ) were much lower than the expected value of one-half, suggesting that the additive genetic factors that contribute to the variance in drug use differ between males and females. Although genetic factors influence male and female drug use to the same extent, the genetic factors that influence adolescent drug use that are shared between males and females are relatively low. Differences between adolescent marijuana and alcohol use also stress the importance of considering the type of drug when examining gender differences in the etiology of adolescent drug use. These data highlight important sex differences in adolescent drug use and suggest that it is important for prevention researchers to recognize that the factors that influence adolescent drug use are not the same for males and females.

## HUMAN LABORATORY STUDIES

Sex-related differences of drugs of abuse have historically received little attention primarily because of the need to control for menstrual cycle phase and FDA restrictions on studying women of child-bearing potential. However, experimental protocols have been developed over the past decade to permit the safe and accurate assessment of acute drug effects in women. In one example, using the prototypical drug cocaine, the protocol was set up to not only study differences between men and women, but to observe differences in the same women during follicular and luteal phases of their menstrual cycle. The experimental framework for such studies has, by necessity, required a multidisciplinary approach so that subjective mood effects are measured in parallel with plasma cocaine levels and physiological responses. Seven male and seven female occasional cocaine users received both an intranasal dose of cocaine hydrochloride (0.9 mg/kg) and placebo powder in a randomized order and reported subjective effects via an instrumental joystick device and various questionnaires. Blood samples were obtained at 5-minute intervals to assess pharmacokinetic differences. Male subjects achieved the highest peak plasma cocaine levels ( $144.4 \pm 17.5$  ng/ml), detected cocaine effects significantly faster than females and experienced a greater number of episodes of intense good and bad effects. Women studied during the follicular phase of their menstrual cycle had peak plasma cocaine levels of  $73.2 \pm 9.9$  ng/ml which was significantly higher than when they were studied during their luteal phase ( $54.7 \pm 8.7$  ng/ml), but there were no differences in their subjective reports of cocaine effects. In spite of the different cocaine blood levels and subjective effects, peak heart rate increases did not differ between males and females, suggesting that women may be more sensitive than males to the cardiovascular effects of cocaine. In a similar experiment during which the cocaine was administered intravenously, the kinetic differences disappeared

suggesting that a difference in absorption was contributing to the findings. These data suggest that there are significant sex-related and menstrual cycle differences in the response to acute intranasal cocaine administration and these differences may have implications for the differential abuse of this drug.

## TREATMENT

Another important area for future research is gender differences in drug abuse treatment services and outcomes. Women's special needs and the delineation of gender specific treatment strategies were first identified over 20 years ago as a result of one of NIDA's initiatives in 1974 supporting research demonstration projects for women's treatment. In the late 1980's and early 1990's, Federal programs such as the NIDA Perinatal 20 and CSAT pregnant and post-partum demonstration grants, also provided support for the expansion and enhancement of women's treatment services and block grant funds mandated "set-aside" for specialized programs/services for women. Although these efforts facilitated development of a gender-specific treatment model that addressed addiction, medical, psychosocial, parenting educational and vocational issues for women and their children, the availability of such gender-specific services remains limited. Of treatment programs in the United States, only 15% of residential programs and 3% of outpatient programs are designed for women only. Moreover, across all modalities (gender-specific and coed), less than one-third (30%) of women who need drug treatment actually receive it.

A lack of attention to gender issues is also reflected in the literature identifying factors related to successful outcomes. This literature suggests a variety of conceptual frameworks such as patient-treatment matching, motivation to change, etc., but all are 'gender neutral'. We know relatively little of the relationship between women's characteristics/needs and treatment outcome. The limited data that are available suggest there are no simple predictors of women's treatment outcomes but rather multiple predictors that reflect complex issues related to medical issues, care-giving responsibilities, child and partner relationships and victimization.

## PSYCHIATRIC COMORBIDITY

One area in which many important differences between male and female substance users have consistently been found is that of psychiatric co-morbidity. A number of studies have demonstrated that anxiety and affective disorders are more common among women with alcohol, cocaine or opiate dependence as compared to men. Male substance users have been shown to have more antisocial personality disorder and more poly-substance dependence as compared to females (Brady *et al.* 1993). One issue of particular importance to gender differences in psychiatric co-morbidity is that of victimization and violence. Victimization, associated with PTSD, as well as other psychiatric disorders, has been shown to be commonly associated with substance use disorders for women in particular. Both epidemiologic and studies of clinical samples demonstrate that for anxiety and affective disorders, the onset of psychiatric disorder precedes the onset of substance use more often in women than in men. These gender differences in order of onset of psychiatric and substance use disorders may have both etiologic significance and implications for gender-specific treatment. If "self-medication" is a more relevant construct for women as compared to men, treating psychiatric disorders may have a differential effect on treatment outcome in women.

There has been little systematic research on gender-specific treatment, but the higher co-morbidity with affective and anxiety disorders in women and, in particular, the difference in order of onset certainly suggests avenues for further exploration. In a large, multisite treatment matching study of various approaches to relapse prevention in alcohol dependent individuals (Project MATCH, 1996), high scores on the psychiatric subscale of the ASI were predictive of a preferential response to cognitive behavioral therapy (CBT) as compared to 12-step or motivational enhancement therapies. Although gender differences in treatment response were not found in the overall data analysis, analysis of specific co-morbid populations has revealed gender differences. In an analysis of the subset of individuals with social phobia in this study, Thevos and colleagues found that women with social phobia had a more robust response to CBT than to other therapies. This was not true for women without social phobia or for men with or without social phobia, suggesting an interaction between gender and psychiatric co-morbidity.

Finally, several studies have shown that pharmacotherapeutic treatment of psychiatric disorders in individuals with co-morbid substance use disorder can improve substance-related outcomes. Cornelius and colleagues (1996) demonstrated that fluoxetine treatment of depressed alcoholics improved both alcoholism and depression. There has been no reported analysis of gender differences in pharmacotherapeutic treatment response for substance using

populations with psychiatric co-morbidity, but a number of studies have shown a more robust treatment response to SSRI's in women as compared to men. Exploration of both pharmacotherapeutic and psychotherapeutic treatment of psychiatric co-morbidity in individuals with substance use disorders may prove to be an important area in investigations of gender-based differences in treatment response.

## **DISCUSSION**

Since 1994, NIH has required that all NIH-funded research involving human subjects must contain females. Since most NIH-funded research, prior to this requirement, largely consisted of male subjects, this requirement was critically important because inclusion of females in a study sample permits the study conclusions to be generalized to females. Such generalization, however, carries the risk of conclusion errors if a gender analysis is not conducted. If a data set contains gender differences, but they are not detected because a gender analysis is not conducted, either of two errors will occur: (1) A conclusion will be drawn that an effect exists, when in fact it exists only for one gender. (2) A conclusion will be drawn that no effect exists, when in fact, (a) it exists in only one gender or (b) there is an effect in both males and females, but in opposite directions, thus negating each other. Conducting a gender analysis, therefore, is a matter of doing good science. Thus, the gender-based research presented in this symposium highlights the scientific importance of conducting gender analyses in drug abuse research. It joins a growing body of drug abuse research that is beginning to suggest that gender differences in drug abuse may be far more pervasive than previously recognized and that recognition and understanding of these differences ultimately can impact on our understanding of the nature of drug addiction, its antecedents and consequences, and prevention and treatment efforts.

## SYMPOSIUM XIV

### TRANSITIONS IN DRUG USE: EVIDENCE FROM GENERAL POPULATION SAMPLES OF ADULT TWINS

*M. T. Tsuang and K.K. Bucholz, Chairpersons*

### CROSS-CULTURAL COMPARISONS OF TRANSITIONS IN SMOKING: EVIDENCE FROM ADULT TWINS IN FINLAND, SWEDEN AND AUSTRALIA

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The study of cigarette smoking is important not only because tobacco use is a major public health problem, but also because of scientific opportunity. Cigarette use is a high prevalence phenotype, even at later stages of dependence. Therefore, the study of cigarette smoking may allow us to address some questions about later stages of dependence that would be much more difficult to investigate in more rarely used drugs of addiction.

In a telephone interview survey of over 6,000 Australian young adult twins, 23 to 35 years of age at the time of their interview, conducted from 1996-2000 (PI, Andrew Heath, AA10249), about 90% of the men and women reported having tried cigarettes, and of those who tried cigarettes about 50% of both men and women reported smoking on a daily basis at sometime in their lives. Unlike alcohol (another substance used by a large proportion of respondents), a substantial number of Australian regular cigarette users reported having experienced problems with nicotine withdrawal.

#### Genetics and Nicotine Dependence

Recent publications on the genetics of nicotine dependence have included an article by Kendler *et al.* (1999), that used a measure of nicotine dependence based on Fagerstrom criteria (Fagerstrom, 1978) administered to adult Virginian female twins; and a second article by True and colleagues (1999) using a measure of dependence based on criteria developed by the American Psychiatric Association (AMA, 1994) administered to US Vietnam-era veteran male twins. Both studies found that more than 50% of the variance in nicotine dependence could be explained by the additive effects of genes, with no evidence for an influence of shared environmental effects. Similar estimates of the proportion of variance due to the additive effects of genes was found for both measures of nicotine dependence in Australian men and women (Lessov *et al.*, manuscript in preparation). These and other findings from the literature provide robust evidence for a strong genetic influence on smoking initiation, persistence in smoking, and for different measures of nicotine dependence.

#### Cross-Cultural Comparisons: Smoking Initiation

How generalizable is the relative importance of genes and experiences shared by family members to smoking behavior across country, age cohort, and sex? To address this question we undertook a set of re-analyses of data obtained from the survey of large numbers of Australian, Finnish and Swedish adult same-sex twins who were in early or middle adulthood at the time of assessment (Madden *et al.*, unpublished manuscript). The Finnish and Swedish twins were both ascertained from population citizen registries, while the Australian twins were a volunteer panel recruited throughout Australia using newspapers and other forms of media. The Australian data used in these analyses were obtained by mailed questionnaire survey in 1980-81; the Finnish data were obtained by mailed questionnaire survey in 1975; and the Swedish data were obtained by mailed questionnaire survey in 1973. Data from these three samples provided information from 11,000 complete pairs of female twins and nearly 10,000 pairs of male twins.

Data obtained from each country were subdivided into 3 age groups--those 18-25 years of age, those 26-35 years of age, and those 36-46 years of age. Despite substantial differences in prevalence across age group and country (from



20% in Finnish women in the oldest age group, to nearly 60% in Swedish women in the youngest age group) and the large numbers of subjects in these samples, we were able to constrain the genetic parameter estimates for smoking initiation (i.e., reporting a history of regular smoking) to be equivalent across age group and society.

However, a stepwise decrease in the contribution from environmental experiences shared by twins was observed going from the youngest to the oldest age group. We may speculate that greater importance of experiences shared by twins in the younger cohorts may relate to changes in the age of onset of smoking. Twins from the younger cohorts, and especially the youngest age group, reported beginning to smoke at a younger age, at a time when it would be more likely that the twins were still spending a substantial amount of time in the company of their brother or sister (and therefore more likely to share environmental influences).

As in the women, we found the magnitude of genetic influences for history of regular smoking in men to be consistent across country and age group. But the magnitude of genetic influence is substantially and significantly greater in men than women; the point estimate in women was 46% compared with 57% in men. Although the pattern for estimates of the proportion of variance in smoking initiation due to shared environmental effects in men were similar to those observed in women—that is, the higher estimate found in the youngest age group of twins—cross-cultural differences were observed in the relative importance of environmental effects shared by family members, with these being more important for the Scandinavian than the Australian men.

### **Genetic Influences: Smoking Persistence**

Using genetic twin analyses we also tested for the importance of genetic influences in smoking persistence defined as continued smoking at the time of assessment. In these analyses, we included pairs where both twins reported a history of regular smoking. More than 50% of the variance in smoking persistence could be accounted for by the additive effects of genes. Unlike smoking initiation, we found no significant differences in the magnitude of genetic influence on maintenance of the smoking habit in men or women, or by age or society.

To what extent do the same genes that predispose someone to become a regular smoker also affect whether or not someone persists in smoking? One structural equation model used to address questions of this nature is the so-called 'correlated liability dimensions model' (Neale and Cardon 1992; Heath *et al.* 1993; Kendler *et al.* 1999), which allows for separate but correlated liability dimensions for smoking initiation and smoking persistence, and therefore, allows the total genetic variance in smoking persistence to be decomposed into components associated with, and unrelated to, genetic influences on smoking initiation.

We fitted this model to our cross-cultural data and tested whether the parameters of the model could be constrained by country, age group, and by gender by likelihood-ratio chi-square test (Madden *et al.*, 1999). Although, we could constrain estimates to be equal across society, we could not constrain our estimates across age group, and this was true in both women and men. In men, the largest overlap in sources of liability for initiation and persistence of smoking occurred in the youngest adults, those 18 to 25 years of age, who on average had the fewest years of cigarette use. A modest 28% of the total variance in persistent smoking in the youngest age group was found due to factors also responsible for onset of regular smoking. However, the percent overlap in liability between early and later stages of smoking dropped to a minimal 7% in later adulthood. Findings in women were completely consistent with those in men for the youngest and the oldest age groups. However, our results in men suggested an increase, rather than a decrease in the proportion of overlap in risk between the onset and persistence of regular cigarette use between those in the youngest and middle age group.

These findings suggest that once someone has become a regular smoker, it may be genetic factors and life events specific to the individual, rather than family background or other shared environmental effects that have the largest effect on maintenance of the smoking habit. The magnitude and sources of overlap between early and later stages of smoking may vary by age and gender. Our results provide substantial evidence for the specificity of genetic influences on liability for smoking persistence, and this is especially true in later stages of adulthood.

## Conclusion

In conclusion, smoking is one of the most strongly familial traits known to science. There is strong evidence for an important role for familial influences at transition to every stage of cigarette use. We ignore familial influences at our peril.

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## TRANSITIONS IN DRUG USE: FINDINGS FROM THE HARVARD DRUG STUDY

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Drug dependence represents the culmination of a series of steps (e.g., exposure to the drug, initiation of use, continuation of use) that must precede the development of dependence. The transition from each level of use to the

next may have different determinants. For example, the transition from never being exposed to a drug to being exposed may be very significantly influenced by the individual's peer group. However, once an individual has developed an abusive pattern of use, the peer group might not have a great deal of influence on the transition to dependence. The various transitions in use probably reflect multiple psychosocial, physiological, and genetic factors.

Using data from the Vietnam Era Twin Registry (VETR) we have previously demonstrated that the probability of becoming a drug abuser is influenced significantly and approximately equally by genetic factors, by environmental experiences shared by both members of a twin pair, and by experiences that are unique to each twin (Tsuang *et al.* 1996). In this paper we will summarize our previous results on influences of genetic and environmental factors on transitions in drug use and present results of new analyses of how certain characteristics of an individual are associated with his probability of making the transitions from one stage of drug use to the next.

Detailed descriptions of the construction of the VET Registry (Eisen *et al.* 1987; Henderson *et al.* 1990) and the Harvard Drug Study (Tsuang *et al.* 1996) have been published elsewhere, so we will provide only a brief description. We interviewed 8,169 male twins (79.7% of those eligible for participation) by telephone using the Diagnostic Interview Schedule Version III Revised (DIS-III-R), a structured psychiatric interview. The mean age of respondents was 44.6 years (S.D.  $\pm$  2.8, range 36 to 55 years), 90.4% were non-Hispanic white, 4.9% were African-American, 2.7% Hispanic, 1.3% Native American/Alaskan Native, and 0.7% "other," and 33.3% reported high school as their highest educational level and 38.6% were college graduates; 92.6% were employed full-time and 1.8% part-time.

Five levels of drug use on a lifetime basis were assessed: 1.) *exposure* (had the individual ever had an opportunity to use the drug); 2.) *initiation of use* (had the individual ever used the drug); 3.) *continuation of use* (had the individual used the drug more than five times); 4.) *regular use* (using the drug at least once per week); and 5.) *substance abuse and dependence* (meeting DSM-III-R diagnostic criteria). The probability of a transition was computed by determining the proportion of those who reached one level of use who went on to the next level of use. The drugs included were marijuana, amphetamine, cocaine, sedatives, heroine, and psychedelics.

A detailed description of our analyses of genetic and environmental influences on transitions has been published elsewhere (Tsuang *et al.* 1999) so results will be briefly reviewed here. About ten percent (10.1%) of twins met the DSM-III-R criteria for abuse or dependence of at least one illicit drug at some time during their lives, with prevalence of abuse or dependence by drug type ranging from 7.2% for marijuana to 1.0% for heroin. The large majority of individuals who met criteria for abuse also exceeded the higher severity threshold for dependence, leading us to collapse these categories. We observed differences among drugs in terms of how likely individuals are to progress from one level of use to another and within any single drug, there are differences among the various transitions. Most individuals who had an opportunity to use marijuana did use it. Most who used it, continued use and most who continued use, became regular users. However, only about one third of regular marijuana users went on to develop abuse/dependence. Cocaine was the only drug for which a majority of regular users developed abuse/dependence.

The following heritability estimates ( $h^2$ ) were derived from biometrical modeling: transition from exposure to initiating use of marijuana,  $h^2=0.44$ ; transition to continued use of marijuana,  $h^2=0.53$ ; transition to regular use of marijuana,  $h^2=0.30$ ; transition from exposure to initiating use of amphetamine,  $h^2=0.61$ ; transition from continued use of amphetamine to regular use,  $h^2=0.39$ ; transition from regular use of amphetamine to abuse,  $h^2=0.61$ ; transition from exposure to cocaine to initiating use,  $h^2=0.54$ ; transition from continued use of cocaine to regular use,  $h^2=0.34$ ; and transition from exposure to heroin to initiating use of heroin,  $h^2=0.50$ . Biometrical modeling indicated that the shared (or family) environment did not make a significant contribution to any of the transitions.

We also examined various characteristics of the individual (serving in Southeast Asia (SEA) during the Vietnam War, major depression, alcohol dependence, antisocial personality disorder, tobacco dependence, and pathological gambling) that might be associated with the probability that a person would make the transition from one level of drug usage to the next level. For clarity in presentation, we refer to these individual characteristics as "risk factors" for the drug use transitions, but it is equally consistent with these data that the drug use behavior could be a risk factor for the individual characteristic. We report the analyses for marijuana use here because marijuana was the

most commonly used drug by our sample and offers the best statistical power for these analyses. Table 1 contains the results of these analyses. Serving in SEA during the war was only related to the transition from initiating marijuana use to continued use, with those who served in SEA less likely to make that transition. Having a lifetime history of major depression was associated with a significantly greater risk of making each transition in marijuana use except the transition from continued use to regular use. Alcohol dependence and tobacco dependence were each associated with a significant increment in risk for every transition in marijuana use. A lifetime history of antisocial personality disorder was associated with the greatest increment in risk for every transition in marijuana use except one (major depression was slightly more strongly associated with the transition to abuse/dependence). A lifetime history of pathological gambling was associated with an increased risk of initiating marijuana use.

Table 1: Odds ratios (and 95% confidence intervals) for the association between risk factors and transitions in marijuana usage.

	Ever Use Marijuana (95% CI)	Use Marijuana > 5 times (95% CI)	Regular Use of Marijuana (95% CI)	Abuse/Dependence of Marijuana (95% CI)
Service in Southeast Asia	1.0 (0.9-1.2)	0.7 (0.6-0.8)	1.1 (0.9-1.4)	1.0 (0.7-1.4)
Major Depression	2.5 (1.9-3.1)	1.5 (1.1-2.1)	1.4 (0.9-2.0)	2.7 (1.8-4.1)
Alcohol Dependence	3.8 (3.3-4.4)	1.7 (1.4-2.1)	1.4 (1.1-1.8)	2.3 (1.6-3.2)
Antisocial Personality Disorder	8.2 (4.3-15.4)	3.2 (1.7-5.9)	2.4 (1.2-4.8)	2.4 (1.4-2)
Tobacco Dependence	2.8 (2.4-3.2)	1.4 (1.2-1.8)	1.4 (1.1-1.8)	1.6 (1.1-2.3)
Pathological Gambling	2.8 (1.5-5.3)	2.3 (0.9-5.3)	1.5 (0.6-3.7)	1.5 (0.6-3.6)

A phenotype such as “DSM-IV drug dependence – present vs. absent” confounds the influences from all of the levels of use that must precede the diagnostic phenotype. When individuals with the diagnosis are compared to those without, the group without the diagnosis includes those who never had an opportunity to try the drug, those who tried it once and never used it again, those who became regular users, but never met criteria for abuse, and those who abused the drug, but never developed dependence. The group, “nonabusers,” is likely to be quite heterogenous for factors that determine membership. For example, some individuals will be nonabusers because they never had an opportunity to use the drug and others will be nonabusers because they tried the drug and found its effects unpleasant.

For each transition, there are probably numerous independent genetic and/or environmentally influenced mechanisms operating. For example, the transition from using heroin more than five times to becoming a regular user might be influenced by the individual’s peer group, by the amount of psychosocial stress that he or she is experiencing and his or her capacity to cope with stress, and by individual differences in the subjective effects of heroin related to the ability to metabolize the drug. Each of these phenomena could be influenced by distinct genetic and/or environmental factors. Therefore, examining transitions does not guarantee that we are studying a simple unitary phenomenon, but transitions are probably less complex and heterogenous phenomena than collapsing levels of abuse, as is typically done.

It might be useful to use transitions to identify “endophenotypes” for genetic research on drug abuse. This would involve identifying a sample of individuals who had an opportunity to make a specific transition and divide the sample into those who made the transition versus those who did not. This approach could be used to identify

characteristics that predicted who made the transition. For example, if the outcome being studied is "drug abuse," the "unaffected" phenotype should be restricted to individuals with the opportunity to become "abusers" who did not become "abusers." Individuals without the opportunity to become "abusers" could be classified "phenotype unknown."

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## DRUG USE TRANSITIONS IN AUSTRALIAN TWINS

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Unlike most psychiatric disorders, substance use disorders are conditional upon exposure to a particular substance. As a result, those who eventually develop substance dependence have progressed through several stages – or transitions - in substance use. These may be broadly classified into the following stages: initiation, casual use, regular or persistent use, problem use, and dependent use. Within each phase, there may be considerable heterogeneity, and the proportion of users who progress through the phases may differ by type of drug, and there may also be different factors that promote or inhibit such progression. The degree to which transitions are influenced by genetic or environmental factors may also vary by drug type and by user characteristics. Elaboration of the various risk factors for each transition has obvious relevance to prevention efforts, a connection that has long been recognized by researchers in the field of adolescent drug use (e.g. Kandel 1985) but there is a paucity of work based on adult samples probing the same sort of transitions, even though the relevance to prevention is as great with adult samples as with adolescent samples. (Warner *et al* 1995). Using data from a community sample of young adult twins in Australia, we describe transitions across drug classes, investigate the influences of comorbid psychiatric disorders on specific transitions, and examine the genetic and environmental influences on transitions.

Data are from a sample of young adult twin members of the Australian Twin Register (ATR) who were interviewed by telephone with a structured psychiatric diagnostic interview between 1996-2000. The twins were registered with the ATR as children by their parents in 1980-82, following appeals through the school system and the mass media. Of the more than 8000 individual twins, 77.4% were interviewed.

For these analyses, only complete same-sex twin pairs were analyzed. Included in this report are 2100 complete pairs (4200 individuals): 698 monozygotic and 513 dizygotic female pairs, and 494 monozygotic and 395 dizygotic male pairs. The interview covered DSM-IV lifetime diagnostic criteria (APA, 1994) for alcohol dependence and abuse, major depression, nicotine dependence, and conduct disorder. History of drug use, including first and last time used, and number of times used were elicited for nine types of drugs: marijuana, cocaine, other stimulants,

sedatives, opiates, hallucinogens, PCP, inhalants, and solvents. For five of these drug classes (marijuana, cocaine, other stimulants, sedatives and opiates), additional information about the frequency of heaviest use of each drug, and six problems associated with the specific drug was obtained. Four problems reflected DSM-IV drug dependence criteria: "using more/longer than intended"; "tolerance"; "use despite drug-caused emotional problems"; and "persistent desire to cut down on use". Two problems applied to drug abuse: "use in situations where could get hurt" and "use interfering with role responsibilities". A proxy measure of dependence/abuse was defined as having at least two problems. Five drug use transitions were defined: ever use, use more than 11 times, monthly (i.e. regular) use, any problems, and 2 or more problems.

Marijuana was the most commonly used drug, followed by stimulants other than cocaine, hallucinogens, sedatives, inhalants, opiates, cocaine and solvents. Altogether, over 60% of the sample had tried at least one of the drug classes, with marijuana accounting for the majority of drug use. Nearly one quarter of those who had ever used marijuana met the modified definition of dependence. Dependence prevalence for the other drugs was much lower than that observed for marijuana. In terms of other psychopathology, about one third met criteria for either DSM-IV alcohol dependence or alcohol abuse, and about the same proportion qualified for a lifetime diagnosis of major depression.

Individuals were classified into the five mutually exclusive transition categories for each drug class. Most were categorized in either the "ever use" or the "two or more problems" categories, with the predominant patterns being ever use and dependent use, with markedly lower proportions in the other transition levels. This pattern was observed across all drug classes. Conditional transition probabilities - transitions that were contingent upon having reached a certain level - were examined. There was great similarity by drug class for the conditional probability of having any problems, and having two or more problems - roughly 80% of those who were regular users progressed to having problems, and about the same proportion had two or more problems. Evidence for greater addiction potential of a certain drug class was not striking, since the conditional probabilities were similar across drug classes. However, there were differences at the less severe end of the drug use transition spectrum, with conditional probability estimates of progressing from ever use to 11 or more times ranging from about a fifth of the sample for most drugs, to nearly half of the sample (for marijuana.)

Multinomial logistic regression, to allow for the five levels of transitions as the dependent variable, was applied to study the influence of other psychiatric disorders on the transitions. The reference group was ever-users. The models were run in STATA (Stata Corp 1997) to take into account the familial nature of the data. Age and gender were included in the model. Analysis of each disorder separately revealed that alcohol dependence increased the odds of every transition in marijuana use. Similar risk elevations for other drug transitions were observed, although in general only the most serious transitions were statistically significant. Alcohol abuse evinced a similar influence, although the magnitudes of the increased risk were lower than those observed for alcohol dependence. Nicotine dependence was observed to have a significant influence on all marijuana use transitions, but its influence on transitions for the other drugs did not achieve statistical significance, with the exception of stimulant dependence. A lifetime diagnosis of major depression had a significant influence on the dependence transition level only, and this was true for all drug classes. The presence of conduct disorder increased the likelihood of each transition in marijuana use, but its influence on transition levels for other drugs was not as marked. Indeed, significant influences were observed only for the most serious transition to two or more problems for each drug class.

When all disorders were included in the model simultaneously, results indicated that alcohol dependence conferred an increase in risk for each transition level for marijuana. Nicotine dependence and conduct disorder were observed to significantly influence transitions to problem use and two or more problems, but no other transitions. Major depression, in contrast, was found to have a significant influence on the severe transition to two or more problems. When transitions for other types of drugs were examined, the only clear pattern to emerge was an elevation in risks at the severe end of the transition spectrum associated with all of the disorders.

One of the objectives of the analyses was to examine whether there were genetic and environmental influences specific to drug dependence vulnerability over and above those for initiation of use. To do this, a two-stage liability model (as opposed to a single stage liability model) was analyzed (See Figure 1). The first stage reflected no use, occasional use and regular use; the second stage reflected non-dependent use to dependent use. Sources of influences are partitioned into genetic influences, and environmental influences of two types - those that are shared by the twins (e.g., being reared in the same family, attending the same schools), and those that are not shared (e.g., attending university, marriage, etc.). As Figure 2 shows, a parameter  $i$  is estimated that reflects the regression of dependence vulnerability on initiation. This parameter may be thought of as the overall shared variance across all influences between stages 1 and 2. The regression coefficient may be thought of as indicative of the degree to which the variance in risk is shared between the two stages. The influences depicted for the second stage (e.g.,  $e'$ ,

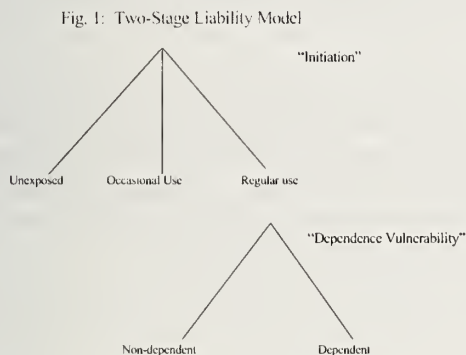
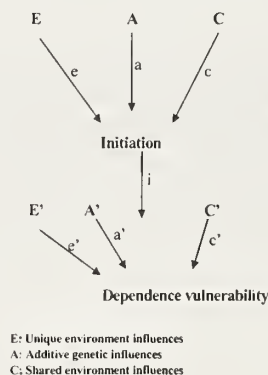


Figure 2: Correlated Liability Model



$a'$ ,  $c'$ ) are those that are specific to that stage. Total amount of variance for Stage 2 may be recovered by simple computation. Our data were ample for computing these models for marijuana and stimulants other than cocaine; there were too few cases of sedative, opiate and cocaine use to compute this model. Models were run using Mx (Neale 1997). Results indicated for both marijuana and stimulants, there was little variance specific to dependence vulnerability. The model also indicated that there were only modest shared environmental influences on initiation. Additive genetic influences significantly explained a substantial proportion of the variances in marijuana and stimulant dependence vulnerability for both men and women. We interpret our data to indicate that there are substantial genetic influences on marijuana and stimulant initiation and dependence vulnerability, but only a negligible proportion of that influence is specific to dependence vulnerability. That is, the genetics of marijuana and stimulant vulnerability appear to be the genetics of regular use of those substances. Furthermore, the contribution of shared environment appears to be quite modest for both drugs. Results were similar for men and women in these analyses.

Several caveats must be borne in mind when interpreting these results. In studying the psychiatric influences on transitions, the temporal ordering of the transition and the occurrence of the disorder was not taken into account. With the exception of conduct disorder, which by definition occurs early in youth, our findings do not permit excluding as a possible interpretation that the psychiatric disorder may have been a consequence of the drug transition, rather than a promoter of that transition. In order to sharpen the interpretation of the results, we will need to investigate onset of the particular disorder in relation to the onset of the specific drug use transition. Another limitation is that our analyses were restricted to same-sex twin pairs, and only those in which both members of the twinship were interviewed. Future analyses will incorporate information from opposite sex twin pairs, and from singleton twins as well.

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## SYMPOSIUM XV

### NEUROIMMUNE EFFECTS OF DRUGS OF ABUSE

*T. K. Eisenstein, M. L. Sopori and M. P. N. Nair*

#### OVERVIEW

The neuroimmunoregulatory network is fundamental to host defense. The activation of the HPA (hypothalamus-pituitary-adrenal) axis by the immune-derived cytokines leads to immunosuppression. It is widely accepted that recreational drugs may act as co-factors in the susceptibility to and progression of HIV infection by acting in synergy with certain HIV proteins to affect the immune and central nervous system of the infected host. The transmigration of infected monocytes/macrophages into the brain through the blood brain barrier may be facilitated by adhesion molecules and their respective ligands on endothelium and monocytes/macrophages.

In this symposium, the participants address the various molecular mechanisms underlying the immuno- and neuro modulation leading to immunosuppression when abusing drugs like morphine, cocaine and nicotine, particularly in diseased state. Dr. Eisenstein presents the role of nociceptin as an immunoregulatory peptide. Dr. Sopori outlines the neuroimmune interactions in nicotine-induced immunosuppression and Dr. Nair presents the neuroimmune effects of morphine and cocaine in AIDS and encephalopathy.

Collectively, these studies show that drugs of abuse bring on an immunosuppression, thereby, increasing the susceptibility to and enhance disease progression in diseases like HIV.

#### NOCICEPTIN AS AN IMMUNOREGULATORY PEPTIDE

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In 1994, the orphan opioid receptor was cloned from cDNA libraries of human, rat, and mouse brain (Wang *et al.* 1994; Bunzow *et al.* 1994; Chen *et al.* 1994; Fukuda *et al.* 1994; Lachowicz *et al.* 1995; Mollereau *et al.* 1994; Wick *et al.* 1994). It has been called the ORL-1 receptor, for opioid receptor-like receptor. It has approximately 65% homology with the classical mu, kappa, and delta opioid receptors. However, it does not bind classical opioid ligands, including naloxone. Like opioid receptors, ORL-1 is a seven transmembrane receptor, is Gi/Go-coupled, inhibits adenylyl cyclase, activates K<sup>+</sup> conductance, and inhibits Ca<sup>++</sup> channels (Meunier 1997). In the year of its discovery, 1994, the presence of ORL-1 in lymphoid tissue was also reported, specifically in rat spleen (Chen *et al.* 1994). Since that time, the presence of the receptor has been reported in other cells and tissues of the immune system including T-cells in murine spleens (Halford *et al.* 1995), human T- and B-cell lines (Wick *et al.* 1995) and monocytic cell lines (Peluso *et al.* 1998), and human polymorphonuclear leukocytes and human peripheral blood mononuclear cells (monocytes) (Wick *et al.* 1995; Peluso *et al.* 1998; Serhan *et al.* 2001). The endogenous ligand for the receptor was reported simultaneously by two groups in 1995 and shown to be present in brains of rats, pigs, and cows (Meunier *et al.* 1995; Reinscheid *et al.* 1995). It was called nociceptin by one group and orphanin FQ by the other. The ligand was shown to be a heptadecapeptide with homology to dynorphin A (Meunier *et al.* 1995). The compound arises from a precursor called either preproorphanin or prepronociceptin (Saito *et al.* 1995; Mollereau *et al.* 1996; Nothacker *et al.* 1996). The precursor molecule of the endogenous ligand has been demonstrated in human spleen cells in addition to neural tissue (Nothacker *et al.* 1996).

The functions of nociceptin have been investigated. In the neural system, nociceptin given i.c.v. induces hyperalgesia (Reinscheid *et al.* 1995; Meunier *et al.* 1995; Mogil *et al.* 1996). It also blocks hyperthermia induced by low-dose morphine, and at high doses, is itself hypothermic (Chen *et al.* 2001). Observations such as these have led to the hypothesis that nociceptin may be an endogenous antagonist to the endogenous opioid system and to morphine. Interestingly, however, when nociceptin is given i.t., it has analgesic properties (King *et al.* 1997). Nociceptin has been implicated in development of tolerance to morphine, as mice with a disruption in the nociceptin receptor gene [knock-out

(k/o mice] have a partial reduction in analgesic tolerance (Ueda *et al.* 1997), and an anti-nociceptin antibody blocks analgesic tolerance (Tian and Han 2000). Other interesting properties of this neuropeptide are that it abolishes the rewarding properties of ethanol in a place-conditioning paradigm, and reduces ethanol consumption in ethanol-preferring rats (Ciccocioppo *et al.* 2000). If given i.c.v., nociceptin induces a rise in plasma corticosterone and augments the cortisone response to the mild stress of a novel environment (Devine *et al.* 2001).

There are only a few papers in the literature addressing the effects of nociceptin on immune function. Nociceptin receptor k/o mice were shown to have normal T- and B-cell numbers in the bone marrow and to have normal immunoglobulin levels in plasma (Nishi *et al.* 1997). However, antisense to the ORL-1 receptor partially blocked the response of normal mouse spleen cells to the microbial mitogen, lipopolysaccharide, as measured by DNA replication in lymphoid cells (mitogen response) and by production of polyclonal IgG and IgM (Halford *et al.* 1995). It has also been shown that human peripheral blood mononuclear cells exposed to the mitogen, phytohemagglutinin, show a ten-fold rise in mRNA for ORL-1 (Wick *et al.* 1995). These observations suggest that nociceptin may be an autocrine factor involved in up-regulation of the immune system. It has also been reported that intradermal injection of nociceptin in the rat increases vascular permeability by release of histamine from mast cells (Kimura *et al.* 2000). Further, nociceptin has been shown to be chemotactic for human polymorphonuclear leukocytes (Serhan *et al.* 2001).

In our laboratory, we have been investigating the effect of nociceptin on the capacity of normal mouse spleen cells to make an antibody response to sheep red blood cells (SRBCs). Spleen cells are dissociated and placed in culture with the SRBCs as antigen (Mishell and Dutton 1967). After five days, the cells are harvested and mixed with an excess of SRBCs plus complement. Monolayers are obtained by introducing the cells into Cunningham chambers (Cunningham and Szenberg 1968). Antibody-forming cells are enumerated by the presence of plaques, areas where antibody has lysed the surrounding SRBCs in the presence of complement. Data are expressed as the number of plaque-forming cells (PFCs)/ $10^7$  spleen cells, with a PFC representing a cell which is secreting antibody to the SRBCs. As shown in Table 1, when nociceptin was added to normal mouse spleen cells, a biphasic dose-response was observed, with maximal inhibition at  $10^{-12}$  M (57%). This experiment has been repeated two additional times with similar results.

Table 1. Effect of nociceptin on the primary *in vitro* plaque-forming cell response

Treatment <sup>a</sup>	No. of PFCs $\pm$ SD <sup>b</sup>	% of Control $\pm$ SD
Control	4017 $\pm$ 349	—
Nociceptin: $10^{-7}$ M	4193 $\pm$ 643	104 $\pm$ 16
$10^{-9}$ M	4280 $\pm$ 602	107 $\pm$ 14
$10^{-11}$ M	4028 $\pm$ 206	100 $\pm$ 5
$10^{-12}$ M	2289 $\pm$ 100	57 $\pm$ 2
$10^{-13}$ M	3297 $\pm$ 239	82 $\pm$ 6
$10^{-14}$ M	3479 $\pm$ 292	87 $\pm$ 7
$10^{-15}$ M	4193 $\pm$ 544	104 $\pm$ 14

<sup>a</sup> Normal spleen cells were incubated with or without nociceptin in Mishell-Dutton cultures for five days.

<sup>b</sup> The number of plaque-forming cells was determined in triplicate cultures for each group.

Additional experiments have shown that the inhibitory effect of nociceptin is not blocked by naloxone at  $10^{-6}$  or  $10^{-8}$  M, an observation which is consistent with reports in the neural system that ORL-1 does not bind classical opioid ligands. In contrast, the nociceptin antagonist, PG-nociceptin (Guerrini *et al.* 1998), at  $10^{-8}$  M (but not  $10^{-6}$  M) blocked nociceptin-induced immunosuppression.

These results imply that splenic leukocytes express ORL-1, and that nociceptin can inhibit antibody responses via this receptor. The target cell cannot be established from this work, as B-cells, T-cells, and macrophages all contribute to the induction of a plaque-forming cell. These studies support a function for nociceptin in regulation of antibody formation.

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## NEUROIMMUNE INTERACTIONS IN NICOTINE-INDUCED IMMUNOSUPPRESSION

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### NICOTINE IS A POTENT IMMUNOSUPPRESSIVE COMPOUND IN CIGARETTE SMOKE

Tobacco is the most abused drug and a major cause of premature mortality around the world (Peto *et al.* 1992). Smoking (SM) significantly increases the incidence of heart disease, cancers of various organs, and respiratory tract infections (reviewed in Sopori *et al.* 1994). It has been postulated that increased susceptibility of smokers to infections and cancer reflects SM-induced impairment of the immune system (Holt and Keast 1977), and SM suppresses the immune system in both animal models and humans (Sopori *et al.* 1994). Several lines of evidence suggest that SM has a major impact on T cell responsiveness; however, despite the decrease in T cell function, the frequency of antigen-binding T cells is not significantly altered in rats exposed to SM (Sopori *et al.* 1993). Therefore, chronic exposure to SM affects T cell responses after the antigen-binding step and may result in T cell unresponsiveness (anergy). To identify the components of SM that modulate the immune system, we demonstrated that continuous administration of nicotine (NT), via subcutaneously implanted miniosmotic pumps for 3-4 weeks, suppressed T cell mitogenesis and the antibody response to T-dependent antigens (Geng *et al.* 1995), suggesting that NT is an important immunosuppressive compound in SM.

### NICOTINE AFFECTS THE ANTIGEN-MEDIATED SIGNALING IN T CELLS

Stimulation of T cells through the T cell antigen receptor (TCR) by an antigen or anti-TCR antibodies initiates a series of biochemical events that may result in T cell proliferation, differentiation, or anergy (Weiss and Littman 1994; Sloan-Lancaster and Allen 1996). The TCR-mediated signal transduction process that leads to T cell activation and proliferation is an increasingly complex array of multiple pathways. However, the major early intracellular events include the stimulation of protein tyrosine kinase (PTK) activities (Weiss and Littman 1994), leading to the activation (tyrosine phosphorylation) of phospholipase C- $\gamma$ 1 (PLC- $\gamma$ 1) that cleaves phosphatidylinositol bisphosphate into diacylglycerol and inositol-1,4,5-trisphosphate (IP3). IP3 acts as a second messenger and increases the intracellular Ca<sup>2+</sup> levels ([Ca<sup>2+</sup>]<sub>i</sub>) by releasing Ca<sup>2+</sup> from the IP3-sensitive Ca<sup>2+</sup> stores and stimulating the Ca<sup>2+</sup> influx (Haverstick and Gray 1993; Clapham 1995). The increased [Ca<sup>2+</sup>]<sub>i</sub> is essential for the cell to progress from the G0/G1 into the S phase of the cell cycle.

T cells from rats treated with NT for three to four weeks, exhibit reduced  $[Ca^{2+}]_i$  levels in response to TCR ligation (Geng *et al.* 1996), indicating that chronic NT affects the TCR-mediated signal transduction pathway at step(s) proximal to the rise in  $[Ca^{2+}]_i$ . Indeed, splenic T cells from chronically NT-treated animals have constitutively stimulated PTK and PLC- $\gamma$ 1, leading to increased basal intracellular levels of IP3 (Geng *et al.* 1996). The constant presence of high intracellular levels of IP3 in NT-treated T cells, we believe, depletes IP3-sensitive intracellular  $Ca^{2+}$  stores (Kalra *et al.* 2000). Because these stores are critical for the communication between the nucleus and cytoplasm (Greber and Gerace 1996; Perez-Terzic *et al.* 1997), the ability of chronic NT exposure to promote depletion of these stores may be a major reason for the immunosuppressive effects of chronic NT exposure (Kalra *et al.* 2000).

## ROLE OF THE CENTRAL NERVOUS SYSTEM IN NICOTINE-INDUCED IMMUNOSUPPRESSION

Increasing evidence suggests a bi-directional communication between the central nervous system (CNS) and the immune system, and the two systems intimately interact during the development, maturation, and aging processes (reviewed in Blalock 1994). These systems may communicate through shared signal molecules such as cytokines and neurotransmitters. While the presence of nicotinic acetylcholine receptors (nAChRs) on lymphocytes is arguable, lymphocytes may respond to relatively high concentrations of NT *in vitro* (Sopori *et al.* 1998), indicating the presence of relatively low-affinity nAChRs. However, chronic administration of relatively very small concentrations of NT into the lateral ventricle caused a significant reduction in the antibody-forming cell (AFC) response that was blocked by the nAChR antagonist, mecamylamine (Sopori *et al.* 1998). These data suggest that some effects of NT on the immune system are mediated through nAChRs in the brain. NT is a classical sympathoadrenal stimulant, and acute NT treatment stimulates the hypothalamus-pituitary-adrenal (HPA) axis, causing secretion of glucocorticoids (Seyler *et al.* 1996). However, our recent results do not support a major role for the HPA axis in the immunosuppression caused by chronic NT exposure, and adrenalectomized rats maintained on constant-release corticosterone pellets displayed immunosuppression that was comparable to sham-operated rats (Singh *et al.* 2000). On the other hand, chronic NT exposure of animals pretreated with ganglionic blockers (chlorisondamine, hexamethonium) prevented the NT-induced immunosuppression (Kalra *et al.* unpublished observation). Thus, the autonomic nervous system may play a critical role in transmitting NT-stimulated immunoregulatory signals from the brain to the immune system.

## NICOTINE IS AN ANTI-INFLAMMATORY

While tobacco smokers exhibit increased risks for several diseases, some diseases are less common in smokers than non-smokers, including ulcerative colitis, sarcoidosis, endometritis, Farmer's lung, and pigeon breeder's disease (reviewed in Sopori *et al.* 1994). Because, most are inflammatory-type diseases, we determined whether NT is an anti-inflammatory. In mice, lethal doses of influenza virus cause severe lung inflammation, leading to acute respiratory distress syndrome (ARDS). To investigate whether NT attenuated the virus-induced ARDS, NT-treated mice were infected with lethal doses of the mouse-adapted influenza A. All the control animals died by day 11, but about 50% of the NT-treated animals survived until day 20, when they were euthanized (Sopori *et al.* 1998). Histopathology revealed that NT treatment significantly reduced the infiltration of leukocytes into the lung tissue (Kalra *et al.* unpublished observation). Moreover, in a model of acute inflammatory response, in which subcutaneous administration of turpentine causes sterile tissue abscess associated with a rise in the deep body temperature (Kozak *et al.* 1997), NT treatment abrogated the rise in body temperature (Sopori *et al.* 1998). These results support the idea that NT is an anti-inflammatory.

## CONCLUSION

Chronic exposure to NT inhibits antibody formation and T cell mitogenesis. This immunosuppression is causally related to constitutive activation of PTKs and depletion of IP3-sensitive intracellular  $Ca^{2+}$  stores. NT may affect the immune system through the nAChRs in the CNS via the autonomic nervous system. Moreover, NT is an anti-inflammatory, which could explain the relative resistance of smokers to some inflammatory diseases.

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## NEUROIMMUNE EFFECTS OF MORPHINE AND COCAINE IN AIDS ENCEPHALOPATHY

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## INTRODUCTION

Although HIV is the etiologic agent for AIDS, there is considerable evidence that cofactors also play a significant role in its pathogenesis. It is now well established that parenteral drug abuse is a significant factor for contracting infection with HIV and subsequently developing AIDS and associated dementia. For several years, we have investigated the premise that drugs of abuse suppress various immune responses that protect the host from HIV

infection and progression to AIDS (Nair *et al.* 1986,1990). We have reported that alcohol, nicotine, morphine and cocaine significantly alter the production, as well as the gene expression of Th-1 and Th-2 derived cytokines (Nair *et al.* 1996,1997,1999). Further, we have shown that cocaine and morphine selectively inhibit HIV suppressing chemokine by lymphocytes from HIV infected patients (Nair *et al.* 2000).

Transendothelial migration of HIV-infected monocytes is a cardinal feature of HIV associated encephalopathy. The transmigration of infected monocytes/macrophages through the blood brain barrier may be mediated by both endothelial and monocyte/macrophage adhesion molecules. Monocytes roll on endothelial cells via inducible adhesion molecules. The rolling monocytes are activated by chemokines to induce integrins that can bind with specific ligands on endothelial cells. Monocytes migration through endothelial cells is mediated by  $\beta$ -1 and  $\beta$ -2 integrins and immunoglobulin supergene family members intercellular adhesion molecules-1 (ICAM-1) and ICAM-2. ICAM-1 is a membrane bound molecule, which is also known to play a significant role in the cell-cell adhesive interaction of the immune system. Elevated levels of soluble serum ICAM-1 have been observed in patients with HIV infection and increase levels of ICAM-1 has been correlated with disease progression (Sipas *et al.* 1993). ICAM-1 has also been shown to be involved in syncytium formation (Hioe *et al.* 1998). Although evidence exists for the role of morphine or cocaine as cofactors in susceptibility to and progression of HIV infection and associated dementia, the molecular mechanisms underlying these effects remain to be determined. We hypothesize that drugs of abuse may increase the production of ICAM-1 by peripheral blood mononuclear cells which may help in the transendothelial migration of infected monocytes into the brain causing neuroimmunopathogenesis of HIV infection.

### Results and Discussion.

Data presented in Figure 1. show the serum levels of ICAM-1 in HIV infected subjects compared to normal subjects. Serum from HIV infected subjects demonstrate a significantly higher level of ICAM-1 ( $478.79 \pm 28.6$  ng/ml  $P < 0.028$ ) compared to  $243.07 \pm 42.96$  pg/mL present in sera obtained from control subjects. Data presented in Figure 2 show the in vitro effect of cocaine on ICAM-1 production by PBMCs from HIV infected and normal subjects. Cocaine at  $10^{-9}$  M ( $P < 0.033$ ) concentration significantly upregulated the ICAM-1 production by PBMC from normal subjects. However in HIV infected subjects, cocaine at lower concentration ( $10^{-12}$  M) produced

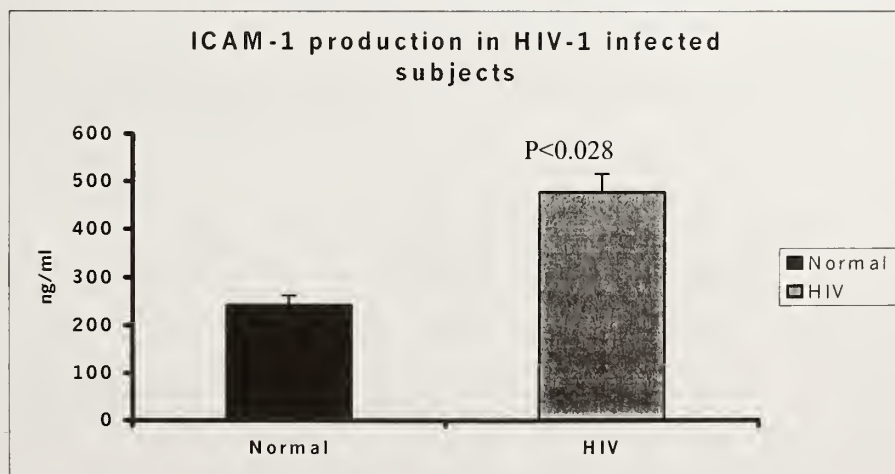
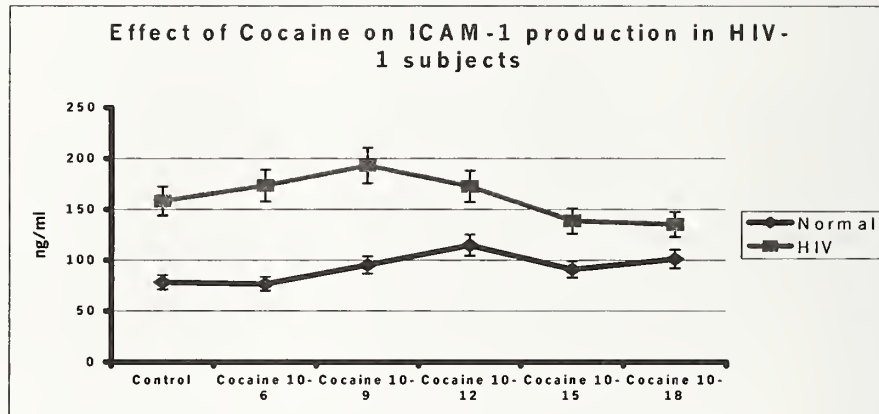


Fig 1 shows the serum levels of ICAM-1 in HIV-1 subjects and age matched controls as measured by ELISA (Biosource Inc). The values represent mean  $\pm$ SD of 10 HIV infected patients and 10 age and sex matched normal subjects. Statistical significance of the differences between HIV patients and normal subjects was evaluated by Student's t test.

significant upregulation of ICAM-1 production ( $p < 0.043$ ). Data presented in Figure 3 show that cocaine and morphine treated whole blood from HIV patients demonstrate increased number of ICAM-1 positive PBMC compared to untreated culture by flow cytometry analysis. PBMC from HIV infected subjects cultured separately with  $10^{-6}$  M cocaine and  $10^{-7}$  M morphine demonstrated 53.1% ( $p < 0.01$ ) and 55.3% ( $p < 0.01$ ) ICAM-1 positive cells compared to 39.6% positive cells shown by untreated culture. In summary, our results demonstrate that HIV infected patients demonstrate significantly higher levels of serum ICAM-1 compared to normal subjects. Further

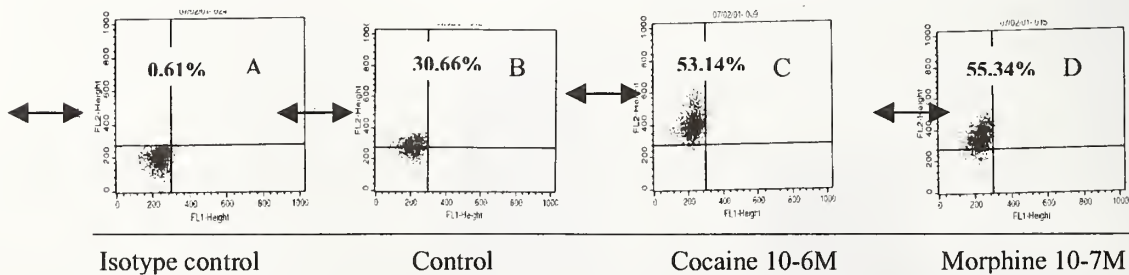
cocaine and morphine can upregulate the production of ICAM-1 by PBMC from normal as well as HIV infected subjects. These studies suggest that drugs abuse may increase the production of adhesion molecules which may help in the trans-endothelial migration of infected monocytes in to the brain causing neuroimmunopathogenesis of HIV infection.



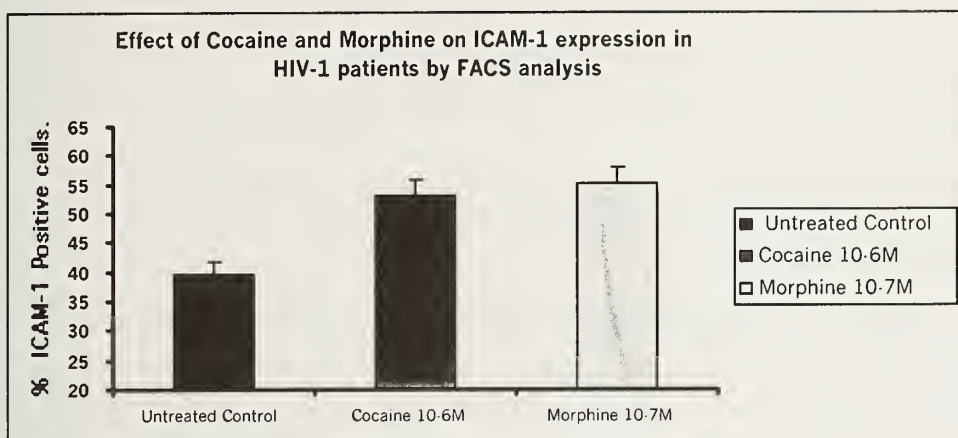
**Figure: 2**

Fig 2: PBMC from HIV infected subjects and age matched controls were cultured with different concentrations of cocaine for 24 hours and the culture supernatants measured for ICAM-1 by ELISA (Biosource Inc). The values represent mean  $\pm$ SD of 10 HIV infected patients and 10 age and sex matched normal subjects. Statistical significance of the differences between HIV patients and normal subjects was evaluated by Students' 't' test.

**Effect of Cocaine and Morphine on ICAM-1 expression by FACS Analysis**







**Figure: 3**

Fig 3 shows the effect of Cocaine and Morphine on ICAM-1 expression in HIV infected subjects by FACS analysis. Panel 3A-D: Shows a FLOW profile for PE- labelled ICAM-1 ( FL-2 axis] positive cells. Panel 3A represents Isotype control, which is used as a negative control to set the quadrant markers. Panel 3B represents Untreated control. Panel 3C represents Cocaine 10-6M treated cells and Panel 3D represents Morphine 10<sup>-7</sup> M treated cells. Panel 3E Shows mean ± SD of 3 separate experiments. Statistically significant difference was observed between cocaine and morphine treated samples as compared to the untreated control by Students' t'-test.

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## SYMPOSIUM XVII

### ADVANCES IN ADOLESCENT TREATMENT RESEARCH

*A.R. Morral and M.L. Deunis, Chairpersons  
J. Flauzer, NIDA, Introductory Remarks*

#### ADOLESCENT DRUG USE: LONG-TERM POST-TREATMENT OUTCOMES

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The therapeutic community (TC) is distinguished from other treatment approaches and other communities by its adherence to "community as method" - the purposive use of the peer community to facilitate social and psychological change. All of the activities of the therapeutic community are designed to produce therapeutic and educational change in the participants, and all participants are mediators of these changes (De Leon, 2000). Adolescents who enter TCs are often at the extreme end of the continuum in terms of antisocial or conduct disorder problems, as well as emotional and psychological distress. They have little motivation to change or to be in treatment, and external pressures are usually required to coerce and to keep him or her there.

As part of a large NIDA-funded study, conducted by the Center for Therapeutic Community Research (CTCR) data were obtained on more than 900 adolescents who entered six TCs (9 sites) in the United States and Canada during the years 1992-1994. The aims of the study were to profile adolescent substance abusers in residential TC treatment, evaluate the effectiveness of the TC for treatment of adolescents, and clarify the relationship between initial admission status, treatment progress and retention. A long-term post-treatment follow-up study (5-7 years) of these youth is nearing completion (e.g., Jainchill, 1997; Jainchill *et al.* 2000). The data presented are from the CTCR baseline interview protocol and a 5-year retrospective time-line interview, conducted at follow-up.

The baseline sample (N=938) was a majority male (76.5%) and Euro-American (49.4%). Most were 16-17 years of age (56%). Almost 56% reported marijuana as their primary drug of abuse, followed by alcohol (20%) and crack/cocaine (9%). More than two-thirds (67.5%) had been mandated to treatment by the criminal justice system. The mean age of first involvement with criminal activity was 11.8 years and 50% reported engaging in violent crimes. The mean age of first use of marijuana was 13.0 years. Eighty-seven percent of the youth had been suspended or expelled from school at least once.

Administration of a structured psychiatric interview revealed that more than 85% of the adolescents entered treatment with at least one non-substance DSM-III-R psychiatric disorder. The most frequently occurring disorders, in order, were conduct disorder (56.7%), oppositional defiant disorder (48.9%), separation anxiety (32.0%), attention deficit-hyperactivity (24.6%), overanxious disorder (23%) and major depression and dysthymia (21.1 % and 20.4%, respectively).

The follow-up sample included all females who were in the baseline cohort and a random sample of males stratified to reflect the program distribution of the baseline cohort (n=715). Sixty-six percent of the sample has completed a 5-year post-treatment interview; 4% have refused, 2% are deceased, almost 3% are pending/scheduled, 1.3% are "out of territory" and less than 1% were considered seriously uncooperative/dangerous. Twenty-four percent remain unlocatable. The few significant differences between the interviewed and uninterviewed sample reflected the deliberate inclusion of all females in the follow-up sample: there were more clients from gender-mixed programs, more females and fewer adolescents who had been mandated to treatment by the criminal justice system. Findings are based on data obtained from 446 5-year post-treatment interviews.

More than half the sample reported some drug and/or alcohol use within the first year of separation from treatment. Hair samples were obtained on 62%; 69% provided urine samples and 55% had both hair and urine specimens. Concordance rates for hair and self-report data ranged between 56.3% for marijuana use and 95.6% for

methamphetamine use. Urine and self-report concordance rates were somewhat higher, particularly for cocaine use (86.1% for urine vs. 68.4% for hair). A fuller presentation of these findings is being prepared for publication.

A cluster analysis of post-treatment lifestyle measures was performed to differentiate clients along a dimension tentatively defined as "lifestyle." Variables selected for inclusion in the cluster analysis described drug use, criminal activity, and prosocial activities and relationships.

Four groups were derived: two prosocial lifestyle types identified as *occasional/non-users* (n=167) and *chronic marijuana users* (n=151); and, two antisocial lifestyle types identified as *drug involved criminals* (n=50) and *criminally involved drug users* (n=64). Differences among the groups were reflected in post-treatment drug use patterns and criminal activity, as well in as prosocial behaviors. For example, there was almost no cocaine use among the two prosocial groups compared with approximately 34% of those in the antisocial groups; and, 20% of *occasional/non-users* and 26% of *marijuana users* reported violent criminal activity, compared with 45% of *drug-involved criminals* and 50% of *criminally involved drug users*. Those in the prosocial groups were also in longer-term relationships, reported more months of employment and/or school and had better family relationships.

The four groups also differed on a number of pre-treatment measures. There were significantly more females and fewer Hispanics among the prosocial groups. Their pre-treatment drug use less often involved cocaine, and they were also less likely to have a diagnosis of conduct disorder.

A significantly greater proportion of those in the prosocial groups had completed treatment (49% and 40%, "*occasionals*" and "*marijuana*" users, respectively) compared with those in the antisocial group (17% and 22%, "*drug involved criminals*" and "*criminally involved drug users*", respectively).

The large majority of the sample (> 70%) indicated relatively prosocial lifestyles for the 5-year post-treatment follow-up period. Their use of drugs was occasional, more regular use was generally restricted to marijuana and they were less criminally involved. Of note, is that there is a significant relationship between completing treatment and post-treatment status described by a prosocial lifestyle.

Historically, attempts have been made to distinguish youth along dimensions of antisocial or conduct disordered behavior. Age of onset, as well as the range and diversity of behaviors, have been shown to differentiate type and severity. In the current sample, the findings suggest a similar pattern of differentiation. At post-treatment follow-up, the 4 groups may best be described by those who use drugs and also commit crimes versus those who are first of all criminals and also use drugs. Whether or not the groups are also clinically distinct remains to be clarified. This understanding, in turn, can guide the development of clinical interventions to address individual needs.

## RELATIONSHIP OF PATIENT SEVERITY WITH TREATMENT PROCESSES AND OUTCOMES AMONG ADOLESCENTS IN DATOS

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The aim of this study is to examine the relationship between treatment processes and post-treatment outcomes among adolescents treated in the Drug Abuse Treatment Outcomes Studies for Adolescents (DATOS-A). We focus on the effects of having a comorbid mental disorder, since previous studies have shown that there is a high prevalence of conduct disorder (CD), attention deficit hyperactivity disorder (ADHD), and depressive disorders among adolescents in drug treatment programs. We expected that youth with comorbid mental disorders would have higher levels of service needs, but lower levels of treatment engagement. Further, we hypothesized that higher levels of treatment engagement and more services received while in treatment would be positively related to post-treatment abstinence.

The DATOS-A study included adolescents treated in 23 adolescent-specific programs in four major U.S. cities from 1993 to 1995 (see Kristiansen & Hubbard, in press). The focus of the present study was on 358 patients sampled from 8 residential programs, 270 patients from 6 short-term inpatient programs, and 182 patients from 9 outpatient

drug-free programs who participated in intake, one-month in-treatment, and 12-month follow-up interviews. The majority of the sample (70%) were male, and 62% were white, 22% were African American, 10% were Hispanic, and 6% were of other ethnic groups. Sixty percent of the sample was aged 16 or older, and approximately 55% were at 10th grade level or higher at the time of admission. Over half (63%) were under criminal justice supervision at the time of treatment admission. Two thirds (66%) met DSM-III-R criteria for marijuana dependence, 37% for alcohol dependence, and 10% for cocaine dependence. Overall, 62% of the sample had at least one comorbid mental disorder; 57% were diagnosed with CD, 14% with depression, and 13% with ADHD.

Analyses consisted of descriptive statistics to describe services needed, services received, treatment retention, and rates of post-treatment abstinence. Log linear models were used to test for the main effects and interactions of comorbidity and modality on services received and treatment processes for categorical variables, and ANOVA was used for continuous measures. Hierarchical logistic regression analysis was used to predict the relationship of services received and other treatment processes to abstinence at follow-up for comorbid and non-comorbid youth separately, after controlling for patient characteristics and type and duration of treatment received.

Services needed and received. Overall, comorbid adolescents averaged a higher number of services needed at treatment admission as compared with non-comorbid youth (means = 4.17 vs. 3.61, respectively,  $p < .01$ ) and received more services while in treatment (means = 1.90 vs. 1.23, respectively,  $p < .01$ ). Comorbid youth had higher rates of 12-step participation while in treatment (84% vs. 74%,  $p < .01$ ), received more individual counseling sessions (3.8 vs. 2.9,  $p < .05$ ), received more group counseling sessions (31.2 vs. 25.1,  $p < .01$ ), and had higher rates of family participation in treatment (40% vs. 22%,  $p < .01$ ) as compared with non-comorbid youth.

Treatment processes and time in treatment. Next, we examined the relationship of comorbidity with the various treatment process measures as reported by the patient. All of these measures showed main effects for modality, but there were no statistically significant differences between comorbid and non-comorbid youth. Similarly, time in treatment did not differ overall by comorbidity, but there was an interaction between comorbidity and modality. A higher proportion of comorbid than non-comorbid youth remained in treatment for at least 90 days in residential programs (71% vs. 64%), but the reverse was true for youth treated in outpatient drug-free programs (28% vs. 49%) ( $p < .01$ ).

Post-treatment abstinence. Overall, abstinence rates were 36% in residential, 22% in short-term inpatient, and 30% in outpatient drug-free programs ( $p = .001$ ). Our previous research with DATOS-A has shown that comorbid youth had a higher likelihood of using marijuana and hallucinogens following treatment compared with non-comorbid youth, although overall abstinence rates did not differ between the two groups (Grella *et al.* in press). In logistic regression models tested separately for the comorbid and non-comorbid groups, two patient characteristics were associated with post-treatment abstinence for both groups. White patients were less likely than African Americans to be abstinent during the follow-up period for the comorbid group (OR = .32, 95% CI = .17, .58,  $p < .001$ ) and for the non-comorbid group (OR = .38, 95% CI = .19, .78,  $p < .01$ ). A higher level of negative peer influence was associated with a reduced likelihood of abstinence for the comorbid group (OR = .83, 95% CI = .74, .93,  $p < .001$ ) and for the non-comorbid group (OR = .81, 95% CI = .72, .92,  $p < .01$ ). Among non-comorbid youth, males were almost half as likely to be abstinent as females (OR = .47, 95% CI = .22, .99,  $p < .05$ ). Variables on type and duration of treatment received were non-significant for both comorbid and non-comorbid youth. Among the variables concerning treatment processes and services, two variables were significant in the model for the comorbid youth. Higher levels of rapport with counselor increased the likelihood of abstinence for comorbid youth (OR = 1.16, 95% CI = 1.10, 1.33,  $p < .05$ ). Similarly, comorbid youth who participated in 12-step groups while in treatment were nearly three times as likely as those who did not to be abstinent (OR = 2.88, 95% CI = 1.22, 6.82,  $p < .05$ ).

As we had expected, adolescents with comorbid disorders had higher rates of perceived service needs as compared with non-comorbid adolescents and received more services while in treatment. Contrary to our expectations, however, there were no differences between comorbid and non-comorbid youth in the degree to which they engaged in treatment. Moreover, only one of the treatment process variables had a direct relationship with post-treatment abstinence, which was counselor rapport for the comorbid youth only. Greater negative peer group influence during the follow-up period reduced the likelihood of post-treatment abstinence for both comorbid and non-comorbid youth. The strongest predictor of abstinence was participation in 12-step groups during treatment among the

comorbid youth, which may have offset the influence of negative peer groups. Number of services received was not an independent predictor of abstinence following treatment for either group. However, the level of service delivery overall was very low and may have been insufficient to produce any detectable treatment effects. These findings indicate that adolescents in drug treatment, both with and without comorbid mental disorders, often fail to receive services specific to their needs, and that the overall level of service delivery in drug treatment programs for adolescents is modest at best.

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## **PREDICTING WHO NEEDS AND GETS MORE TREATMENT AFTER INITIAL ASSIGNMENT TO OUTPATIENT TREATMENT**

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Substance use is a condition characterized by chronic relapses, which often requires multiple treatment episodes before an adolescent reaches recovery. Between 20% to 50% of adolescents entering treatment have previously been in treatment. After discharge from the initial outpatient treatment episode, over half of the adolescents are commonly transferred to another level of care, referred to more care, or readmitted within 12 months (Dennis, Dawud-Noursi, Muck, & McDermeit, in press). The Cannabis Youth Treatment (CYT) study demonstrated the effectiveness of 5 types of short (6-14 week) outpatient treatments, but over two-thirds of the adolescents still needed more treatment (Dennis *et al.* 2000). The goals of this presentation are to a) describe the pattern of post-treatment recovery, b) examine the pattern of additional treatment and its relationship with recovery, and c) determine the predictors of who needs and gets additional treatment.

CYT involved 600 adolescents with marijuana disorders who were appropriate for and randomly assigned to one of five outpatient treatment approaches (Dennis, Titus *et al.* in press). One of the largest studies ever conducted on adolescent outpatient treatment, CYT involves a multi-center collaboration of the Center for Substance Abuse Treatment (CSAT), two of the U.S.'s largest providers of adolescent treatment (Operation Par in Florida and Chestnut Health Systems in Madison County, Illinois), and two of the U.S.'s major medical centers (University of Connecticut Health Center in Farmington, Connecticut, and Children's Hospital of Philadelphia, Pennsylvania) as well as a coordinator center (led by Chestnut Health Systems in Bloomington, Illinois). Follow interviews were completed on over 94% per quarter for 12 months (89% with all 4 interviews). Clinically, 85% used marijuana before age 15, 71% used at least weekly, 46% and 26% had been in treatment before. This is as or more severe than similar adolescents in the U.S. public treatment system (Tims *et al.* in press). Demographically, 87% were in school, 85% 15 or older, 83% were male, 62% in the criminal justice system, 61% white, and 47% employed.

About a third of the adolescents were in recovery (i.e., no past month marijuana use or substance use disorder problems while living in the community) at each follow-up but many changed their status between interviews. By month 12, 9% had sustained their recovery, 39% had a sustained pattern of problems, and 52% changed their recovery status one or more times (15% who recovered after month 3 and sustained it; 7% who had intermittent periods of recovery and ended in recovery, and 29% who had intermittent periods of recovery and ended with problems). If we look at those who initially responded to treatment (at month 3), 29% sustained their recovery through month 12, 22% had some relapses but were in recovery at month 12, and 49% had relapsed and continued to have problems at month 12. Of the people who were not initially in recovery at month 3, 23% later achieved and sustained their abstinence, 20% had periods of abstinence but had relapsed by month 12, and 57% never achieved a recovery during the period. Thus, the odds of being in recovery at 12 months were 3.69 times higher ( $p < .0001$ ) for those who were initially in recovery, but both groups continued to be in a state of flux. The odds of being in recovery at 12 months were 1.28 times ( $p < .05$ ) more likely for those who received additional treatment (30%) than those who did not. The statistically significant predictors of who received subsequent treatment were adolescents who had previously been in treatment (odds ratio=1.84), were under age 15 (1.85), above the median on behavior problems (1.72), and who still had substance use disorder symptoms at discharge (1.11 per symptom). The

statistically significant predictors of who was in recovery at month 12 were adolescents who were female (1.66), had a family history of substance use problems (0.56), continued to use marijuana at month 3 (0.98 per day of use), were in recovery (no use or problems while in the community) at month 3 (2.43), and days of post CYT treatment (1.03 per week of additional treatment).

Recovery is clearly a dynamic state that changes over time in both directions. Early response to treatment and subsequent treatment were associated with being in the better outcome groups. While better than existing practice, the CYT interventions were not an adequate dose of treatment for over two-thirds of the adolescents. It is essential to conduct aftercare monitoring with all adolescents (even those who initially do well). Only a fraction of those in need of more treatment received it, so there is a need for more pro-active continuing care services.

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## **DRUG ABUSE OUTCOMES AS THE ADOLESCENT TRANSITION INTO YOUNG ADULthood**

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One area of adolescent health that is under-studied is the clinical course of substance use disorders. Such knowledge would inform models on treatment effectiveness and maintenance of behavior change. Many research questions remained unanswered, particularly with respect to the relationships between a reduction in substance use and positive change in functioning among adolescents who undergo substance abuse treatment.

We earlier reported on the one-year clinical course of adolescents who had received drug abuse treatment. Fifty-three percent of treatment completers reported abstinence or only a minor lapse (defined as infrequent use of drugs and absent of drug-related consequences) (Winters, *et al.* 2000). Among those who did not complete treatment, 15 % reported this outcome pattern. Thus, non-problem drug use or abstinence was observed in an appreciable percentage of drug-abusing teenagers who had earlier sought treatment. The purpose of this study was to investigate the clinical course of the sample over an extended period. Based on our previous research and similar work of others (e.g., Maisto *et al.* 2001), we hypothesized that at six-year, (i) drug use level and prevalence of a substance use disorder (SUD) will be reduced in the drug-clinic group compared to their levels observed at one-year, (ii) however, drug use level and prevalence of a SUD will be higher in the drug-clinic sample than in a non-drug-clinic comparison group, and (iii) treatment utilization during the intervening period will reduce the likelihood at six-year of having a SUD.

Participants. Drug clinic participants were 245 adolescents who were originally assessed, when age 14-18, at which time they had a current DSM-IV SUD diagnosis and were recruited from a chemical dependency treatment program. Six-year follow-up data were available at the time of this presentation for 193 of the 206 adolescents who had reached their six-year assessment anniversary (thus producing a 93% follow-up rate among the 206 eligible subjects). Non-drug-clinic participants were 135 adolescents from the community who were recruited as part of an adolescent health study of normal development. These comparison subjects were matched to the drug clinic group for gender, ethnicity, age and SES. Six-year follow-up data were available at this time for 100 of the 109 adolescents who had reached their six-year assessment anniversary (which is a 92% follow-up rate among the 109 eligible subjects). The mean age of the two groups at six-year were 22 years and 23 years, respectively.

Procedure. Drug clinic participants completed a detailed assessment protocol at baseline and one and six year follow-ups that addressed substance use, substance use disorders, and several other domains including psychosocial functioning, comorbid psychopathology, health, and treatment utilization. A family informant, who was most often the participant's biological mother, completed an interview and questionnaire at the same three assessment points. Urine was collected at one and six year follow-ups up to test for the presence of alcohol, THC at 20mg/ml,

amphetamines, opiates, barbiturates, cocaine, and benzodiazepines. The normal comparison participants completed a comparable assessment protocol at baseline and six-year follow-up.

Measures. Substance use frequency (an aggregate measure created by summing across 12 drug categories, range 0-72) and psychosocial functioning were assessed with the Personal Experience Inventory (PEI; Winters & Henly, 1989), SUD and comorbid psychopathology were measured with the Adolescent Diagnostic Interview (ADI; Winters & Henly, 1993), and health and treatment utilization were assessed with a supplemental interview. Adaptations of these instruments were made for the one and six year follow-ups. The focus of this paper is on the substance use frequency and SUD data at the one- and six-year data points, and the treatment utilization data at the six-year follow-up.

Course of drug use level and SUD, drug-clinic group. For the drug-clinic participants, we computed a prior year aggregate drug use frequency (DUF) score for the one- and six-year data points. We also categorized these participants into two SUD groups: No SUD or Any SUD. Consistent with the hypothesis, a one-way ANOVA with repeated measures (one- and six-year data) found that there was a statistically significant reduction in DUF across time ( $p < .01$ ). To examine changes in SUD groups across time, we computed a kappa statistic and found it to be quite low (.24). An examination of the data indicated that there was a significant reduction in Any SUD from one-year to six-year (from 48% to 29%) (and thus a significant increase in No SUD from one-year to six-year was found as well).

Drug-clinic versus normal comparison. This analysis, which compared the drug-clinic and normal comparison groups, focused on six-year outcome. As expected, the one-way (group) ANOVA revealed that the drug-clinic group had higher levels of prior year DUF compared to the normal comparison group ( $p < .01$ ). A chi square analysis revealed that the two groups differed significantly in terms of any SUD versus no SUD ( $p < .01$ ). The prior year rates of any SUD rates were 29% (drug clinic) and 11% (comparison).

Treatment utilization and SUD. The final analysis examined the likelihood of a SUD being absent at six-year when treatment or therapeutic support occurred during the one- to six-year follow-up period. This analysis focused on drug-clinic participants. Three treatment variables were examined with the odds ratio statistic: received formal drug treatment, received formal mental health treatment, and participated in AA (more than two meetings). All of these treatment variables produced a significant odds ratio ( $p < .01$ ), indicating a greater likelihood of a prior year SUD being absent at six-year in the presence of some treatment occurring. The obtained odds ratios were as follows: received drug treatment, 2.3; received mental health treatment, 1.8; involved with AA, 2.5.

The findings for six-year outcomes were consistent with the three hypotheses. Drug-clinic participants showed a significant reduction in overall drug use frequency and in the rate of SUD at the six-year follow-up compared to the findings from the one-year follow-up. However, the six-year outcomes in terms of level of drug use and rate of SUD were significantly higher than the findings obtained from a comparison normal group. The findings also supported the possible mediating role of additional treatment or therapeutic experience with respect to the course of a SUD. We found that the use of treatment services during the prior 5 year period, whether it is drug- or mental health-related, increased the chances of not having a SUD at the six-year data point.

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#### **PHOENIX ACADEMY TREATMENT OUTCOMES: PRELIMINARY FINDINGS FROM THE ADOLESCENT OUTCOMES STUDY**

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There is a small but growing literature on the effectiveness of adolescent drug treatment interventions. Unfortunately, few rigorous studies evaluate the effectiveness of the treatments most commonly available to youths,

their families and referring agencies. Instead, the adolescent treatment literature divides neatly between rigorous evaluations of novel theory-driven approaches that are not currently available in community settings, and evaluations of existing programs that use research designs too weak to support strong conclusions about program effectiveness. Typically, studies report treatment outcomes of youths who enter, for instance, inpatient versus outpatient community programs, without accounting for known differences in problem severity and other pretreatment characteristics of youths who enter these treatment modalities. Although statistical approaches are available to adjust comparison groups to account for known differences in background and drug use, we are aware of no published analyses of the major community treatment studies that use these approaches to generate more valid comparisons of treatment outcomes. This may be because the differences between treated cohorts are too extreme to be legitimately remedied through statistical adjustments.

In this report, we describe the treatment outcomes of 449 juvenile probationers referred by Los Angeles Probation to Phoenix Academy of Lake View Terrace, or to one of six group homes of comparable size and planned treatment duration, but which offer no specialized or intensive drug treatment services. Additional information about the group homes and study design are published elsewhere (Morrall *et al.* in press). Sequential referrals to these seven placements were recruited for study participation between February 1999 and April 2000, if they were between the ages of 13 and 17, if they assented to participate and to having their parents notified of participation. These procedures resulted in a sample of 175 youths admitted to Phoenix Academy (the PA condition), and 274 comparison youths who entered other placements (COMP condition). Youths were interviewed using the Global Appraisal of Individual Needs (Dennis, 1998) while in detention prior to placement, and again 3, 6 and 12 months later. Over 90% of the baseline sample was successfully interviewed at each follow up.

In aggregate, participants were 87% male, 55% Latino, 16% white and had a mean age of 15.5 at the initial interview. A large percentage acknowledged symptoms qualifying them for either substance dependence disorders (53%), or substance abuse disorders (26%). Collectively they acknowledged committing 11,272 crimes in the 90 days preceding the initial interview. Nevertheless, substantial group differences distinguished PA and COMP participants. Indeed, among the 55 baseline variables we selected, a priori, as the most important indicators of group comparability (the "key variables"), significant differences were observed on 26.

To improve the comparability of the COMP and PA conditions, Propensity Score Analysis was used to weight COMP cases (Rosenbaum & Rubin, 1985). In the first stage of this case-mix adjustment approach, 24 of our 55 key variables were entered as independent variables in regression with treatment condition as the dependent variable. We used a boosted logistic regression algorithm (Friedman, *et al.* 2000; Ridgeway, 1999) to estimate the non-linear relationship, including up to three-way interactions, between the key variables and the treatment condition. The predicted values from this model correspond to the model estimates of the probability the case belongs to the PA condition. Case weights for the COMP condition were constructed as the odds associated with each of these probabilities. After applying weights to the COMP condition, only 4 significant mean differences remained between conditions on any of our 55 key variables: 1) PA youths had higher scores on a scale assessing the degree to which they attribute their problems to drugs (Problem Orientation Index: PA=1.38, COMP=.98); 2) a higher proportion of COMP youths denied ever using any drug or alcohol (PA=.00; COMP=.01); 3) a higher proportion of the PA sample reported a treatment need for drugs other than marijuana or alcohol (PA=.32, COMP=.20); and 4) COMP youths reported more recent engagement in illicit activities other than drug use.

Treatment outcomes were assessed with a between-groups repeated measures analysis for which COMP case weights were applied. At each interview, youths were asked how many of the past 90 days they spent in a controlled environment. Preliminary analysis confirmed that each group had equivalent mean times in controlled environment at each survey wave. Therefore, responses to this item were incorporated as a time-varying covariate in the hierarchical linear model of our repeated measures analyses, since outcomes like drug use must be adjusted for time "at risk." Drug use, crime and psychological functioning outcomes were assessed.

The analysis results showed a consistent pattern across many outcomes. For drug use, crime and psychological outcomes, PA and COMP participants had similar problems at baseline, and comparably sharp reductions in problems three months later. From that time on, however, PA problems remained stable or continued to decline, whereas COMP problems generally increased. In many cases, these trends reflected statistically significant time by treatment condition interactions. Significant group by time interactions were found for the Substance Frequency



Index ( $F[3, 1192]=3.5, p<.05$ ), a measure of the frequency and intensity of recent drug use, the Substance Problem Index ( $F[3, 1192]=4.7, p<.01$ ), a count of the number of dependence and abuse symptoms experienced in the past month, and the Anxiety Symptoms Index ( $F[3, 1192]=3.5, p<.05$ ), a measure of recent anxiety symptoms. The same patterns of outcomes with nearly significant group by time interactions were found for property crimes ( $F[3, 1192]=2.16, p<.10$ ), and somatic symptoms ( $F[3, 1192]=2.2, p<.10$ ).

These results suggest that therapeutic community treatment for adolescent substance abusers is associated with greater reductions in subsequent drug use, drug use problems, and some psychological distress than are found among comparable youths who receive residential interventions of similar duration and intensity, but without intensive substance abuse treatment services. Because this study used a quasi-experimental design, rather than random assignment to treatment condition, we cannot unequivocally attribute the observed differences in treatment outcomes to the treatment programs themselves. Unmeasured group differences might explain differences in outcomes better than treatment effects. Nevertheless, because this study employed a seemingly successful case-mix adjustment strategy, it offers a more rigorous examination of treatment effects than has been the norm in evaluations of community-based treatments for adolescents.

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## ADVANCES IN FAMILY-BASED THERAPY FOR ADOLESCENT SUBSTANCE ABUSE

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Treatment research in the adolescent substance abuse specialty has evolved rapidly in the past decade. Family based therapies have been among the strongest performers in the outcome studies on adolescent drug abuse (Ozechowski & Liddle, 2000). Reviews have concluded that certain forms of family based therapies have produced the strongest available evidence in the amelioration of teen drug use (e.g., Williams & Chang, 2001). This presentation summarizes treatment findings on empirically supported family based treatment for adolescent drug abuse – multidimensional family therapy (MDFT) (Liddle, 2001).

The efficacy of MDFT was examined in comparison to individual adolescent treatment – Cognitive Behavioral Therapy (CBT). This study is noteworthy because of the comparison it provides - it is one of the first adolescent drug abuse studies comparing family therapy to a state-of-the-art psychotherapy. Additionally, this study has many design and analysis features expected in high quality intervention studies (e.g., DSM diagnosis on all subjects, manualized interventions representing commonly applied treatments (family and individual treatment) extensive manual adherence analyses, state of the art measures, multiple measures of adolescent outcome, state of the science statistical methods, true intent-to-treat design, comparison of two theory-derived strong treatments, and control of intervention dose). Two hundred twenty-four adolescents referred to a community clinic for substance abuse treatment were randomly assigned to one of the two treatments. The final sample was primarily male (81%), African American (72%), juvenile justice involved, and low income (e.g., 38% report total yearly family incomes of less than \$10,000; 23% between 10,000-20,000). Self-reported adolescent drug use, and adolescent-reported and parent-reported externalizing and internalizing symptomatology, were assessed at intake and again at 6 and 12 months following treatment termination.

As we found in an earlier study (Liddle, *et al.* 2001), MDFT was successful in reducing marijuana use (linear slope effect  $t = -3.94, p < .001$ ), drug involvement (linear slope effect  $t = -5.82, p < .001$ ), as well as externalizing (parent report  $t = -6.09, p < .001$ ; youth report  $t = -4.05, p < .001$ ) and internalizing symptoms (parent report  $t = -3.72, p < .001$ ; youth report  $t = -2.46, p = .014$ ). Thus, the significant linear rate of change was present for each of the outcomes indicating that the shape of the change is linear and negative, suggesting improvement. CBT was likewise effective for drug involvement (linear slope effect  $t = -3.19, p < .002$ ), and parent report of externalizing ( $t = -2.81, p = .005$ ), and internalizing symptoms ( $t = -3.27, p = .001$ ). However, the shape of the change was not linear over time

for certain outcome variables. For example, the linear effect was not significant for marijuana use, adolescent report of externalizing symptoms and adolescent report of internalizing symptoms. For the CBT treatment group, there is a general leveling off in marijuana use after the 6-month follow-up.

After conducting the analyses within the treatment conditions, we examined the findings between conditions by employing a Level 2 equation. There were no significant differences between conditions in the rate of change over time with respect to marijuana use, parent report of the youth's externalizing symptoms, and youth report of internalizing symptoms. A significant difference between treatment conditions for the linear slope was observed for the Personal Involvement with Chemicals scales of the PEI ( $t = 2.29, p = .022$ ). Adolescents receiving MDFT in comparison to youth who received CBT continue to improve after termination as measured by the PEI, Personal Involvement with Chemical subscale. For externalizing symptoms, there was a significant difference between treatment conditions on parent's report of their child's externalizing symptoms ( $t = 2.07, p = .038$ ) with adolescents receiving MDFT continuing to improve after termination, and adolescents in the CBT condition showing an leveling off of symptom reduction. Finally, with respect to internalizing symptoms, there was a significant between treatment difference with respect to adolescent report of their symptoms, with youth in MDFT condition reporting continued improvement after treatment; while adolescents in the CBT condition appear relatively stable after suspension of treatment ( $t = 2.29, p = .022$ ). Lastly, we examined whether any demographic variables (adolescent age at intake, gender, race, criminal justice involvement, family structure, family income, mother's education) added to Level 2 would act as an important covariate to treatment condition. None of these variables improved the explanatory power of the basic hierarchical models already discussed.

Considering the results as a whole leads us to conclude that in this comparison of two state-of-the-art treatments for adolescent substance abuse, as expected, both treatments emerged as at least somewhat efficacious. Both treatments reduced symptomatology from intake to termination across all three domains of functioning: drug use, externalizing symptomatology, and internalizing symptomatology. However, while both treatments were efficacious from intake to termination, the treatments show different long-term trajectories. The rate of improvement of symptoms between the two treatments is different such that only MDFT was able to maintain the symptomatic gain after termination of treatment. MDFT shows a significantly different slope from CBT suggesting that youth who received MDFT continued to evidence treatment improvement after termination. The advantage to MDFT, then, concerns its ability, in comparison to CBT, to retain the effects of treatment beyond the treatment phase.

It is important to recognize that these results were achieved with two theoretically different but standard psychotherapies. The models tested here are traditional psychotherapeutic interventions provided in standard service delivery formats. The treatments were both clinic based therapies providing once a week face-to-face therapy with no booster sessions. The fact that improvement in symptomatology was found in such modest dose treatments delivered to such a challenging patient population given its risk exposure and level of initial dysfunction is an important indicator of the promise of CBT (in terms of immediate therapy effects) and especially MDFT (in terms of immediate and continued effects at one year post termination) effects in the treatment of adolescent drug abuse.

Although the data show efficacy, there is room for improvement. The success of multiple systems focused therapies, with their intensity of service delivery, case management components, and home-based service delivery contexts, leads us to speculate that improved outcome would be achieved by integrating the psychotherapeutic models tested here into a more multisystemic service delivery context which includes case management, face-to-face therapy sessions of more than once per week delivered in the home if necessary. One of our current controlled studies is testing our most intensive and extensive version of MDFT developed to date.

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SYMPOSIUM XVIII  
HALLUCINOGENS AND RELIGION: HISTORICAL TO SCIENTIFIC PERSPECTIVES

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In the United States, hallucinogens are classified as Schedule I substances under the Controlled Substances Act because they are considered to have high abuse liability and to be of no therapeutic value. The Schedule I classification gives rise to the common assumption that the hallucinogens have no value of *any* type, or no net value relative to their risks. Yet psychoactive plants having hallucinogenic effects have been valued for thousands of years in many cultures, in structured contexts, for their ability to facilitate spiritual (i.e., mystical/transcendent) experiences (Schultes and Hofmann 1992). The phenomenology of such mystical experiences has been well-described and, as discussed below by Dr. Hood, can be reliably measured. Many scholars of religion believe that “naturally-occurring” mystical experiences, often occasioned by prayer, fasting, solitude or other austerities, have provided the bedrock phenomenological foundation for most of the world’s religions (Smith 2000). That is, the founders of many religions had profound mystical experiences on which they based their teachings.

Of relevance to this symposium is the observation/hypothesis that, under appropriate conditions, hallucinogens can occasion profound mystical experiences that are indistinguishable in description and impact from the “naturally-occurring” mystical experiences. The striking similarity between the drug-occasioned and austerity-occasioned mystical states suggests the intriguing possibility that the two may be mediated by common biological mechanisms. In fact, the last several years have witnessed increasing interest in the neurobiology of mystical experiences (Austin 1999; Newberg *et al.* 2001). A basic premise of this growing field of “neurotheology” is that the compelling commonalities among mystical experiences reported across time and across different cultures and faiths suggest a common neurobiology reflecting the structures and function of the human brain.

As discussed in this symposium on hallucinogens and religion, use of these drugs in natural settings has been studied from the perspective of anthropologists and historians. The preclinical and clinical behavioral pharmacology of these drugs have also been studied in laboratory contexts quite different from the religious contexts in which these drugs are known to be used. However, a vast gap exists between our knowledge of these drugs obtained using the descriptive methods of anthropology and the knowledge obtained using modern clinical pharmacology methods. The gap is even larger than it would be otherwise because, largely in reaction to the excesses of the 1960s “psychedelic movement,” there has been virtually no human research with hallucinogens for the last thirty years.

The papers in this symposium discuss the use of hallucinogens in spiritual practices from several different perspectives. Dr. Charles Schuster, a behavioral pharmacologist, discusses the neurochemistry and preclinical and clinical pharmacology of hallucinogens, and makes the point that the classic serotonergically-mediated hallucinogens such as LSD, mescaline and psilocybin do not show classic abuse liability as evidenced by self-administration in animals and euphoria in humans. Dr. Marlene de Rios, a medical anthropologist with particular expertise on the use of ayahuasca in South America, considers hallucinogens from historical and cross-cultural perspectives, commenting on the socially important roles that hallucinogens have had in some cultures and the apparent lack of abuse potential when used under careful ritual circumstances. Bob Jesse, president of the Council on Spiritual Practices, which is sponsoring research on primary religious experience, then considers contemporary perspectives on hallucinogens in spiritual practices, discussing nomenclature, pivotal observations of several modern scholars, and a rationale for further exploration in this area. Dr. Ralph Hood, an expert in the measurement of the mystical experience, discusses the development of the contemporary science of religion, describing the development of reliable and valid measures of the mystical experience which are essential for advancing the scientific analysis of hallucinogen-occasioned mystical states and their impact on spiritual practices. The symposium concludes with discussion comments from Dr. Herbert Kleber, a psychopharmacologist with expertise in drug abuse policy and who has conducted human clinical research with hallucinogens. This multidisciplinary symposium represents a renewed integration of human religious and spiritual experiences into the domain of scientific investigation.

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## PRECLINICAL AND CLINICAL PHARMACOLOGY OF PSYCHOACTIVE DRUGS USED IN SPIRITUAL PRACTICES

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The psychoactive drugs that are used in conjunction with religious ceremonies and for other self-actualizing purposes have been studied, revered, and vilified by individuals with different perspectives and values. Chemically these agents fall into three general classes: the phenylalkylamines (phenethylamines); the indolealkylamines (tryptamines); and the beta-carbolines. They are generally termed by pharmacologists "classical hallucinogens" because they share a similar profile of pharmacological actions in both laboratory animals and humans. It should be noted, however, that the psychoactive effects of these agents are modified by the set of the individual who ingests the drug and the setting in which it takes place. With repeated administration at short intervals tolerance develops to many of their psychoactive and physiological effects. Tolerance to one of these drugs generally conveys cross-tolerance to other members of this group, evidence of their pharmacological similarity (e.g., Wolbach *et al.* 1962). Further evidence of pharmacological similarity is the recent neurochemical studies indicating that the same serotonergic receptor sub-type (5-HT<sub>2a</sub>) in the brain (Glennon *et al.* 1984; Ismaiel, *et al.* 1993; Winter *et al.* 1999) mediates their psychoactive effects.

Since none of the classical hallucinogens have accepted medical use in the United States and are considered to have "abuse potential," they have been placed in Schedule I of the Controlled Substances Act. Other widely abused drugs such as heroin and phencyclidine are also in Schedule I. However, there is considerable evidence demonstrating that the "classical hallucinogens" can be differentiated from these other commonly abused drugs in several important ways. It is well established that drugs that are commonly abused by humans will serve as positive reinforcers in a wide variety of animal models of drug abuse (Johanson and Balster 1978; Griffiths *et al.* 1979). In contrast, none of the "classical hallucinogenic" agents have been shown to be self-administered by animals (Deneau *et al.* 1969). This is due to the fact that the classical hallucinogens have a different profile of pharmacologic effects on the monoamine systems of the brain compared to drugs such as heroin, cocaine, and alcohol. In fact, the limited evidence that exists suggests that the classical hallucinogens are aversive in animals and will serve as negative reinforcers (Hoffmeister 1975). This is in accord with the observations of humans ingesting drugs such as mescaline, psilocybin, or the ayahuasca tea (containing DMT and the beta-carbolines) who frequently show nausea, vomiting, or diarrhea and rarely the type of euphoria associated with drugs of abuse (e.g., Grob *et al.* 1996).

The substituted amphetamines, MDMA and its relatives such as MDA and MDE (a sub-class of the phenethylamines) have a unique pharmacology. They not only share certain actions with the stimulant amphetamines but also with the "classical hallucinogens." In humans, MDMA and its relatives are purported to have an "empathogenic" effect, which has been claimed to facilitate psychotherapy. There have been no well-designed clinical trials conducted to demonstrate the efficacy of MDMA in facilitating the effects of psychotherapy. Nevertheless, the reports of its empathogenic effects have led to its use by people striving to gain a feeling of greater connectedness and brotherhood with fellow humans. However, in contrast to the classical hallucinogens, the substituted amphetamines are self-administered by laboratory animals (e.g., Beardsley *et al.* 1986; Griffiths *et al.*

1979; Sannerud *et al.* 1996). In drug discrimination studies, these drugs have been shown by some investigators to substitute for amphetamine but in other studies they fail to do so. Further, in some studies, they substitute for one of the classical hallucinogens and in others they do not. These results are most likely attributable to differences in the training doses of the drugs used, as well the training procedures. Nonetheless, they illustrate that these agents have mixed effects. Recent drug discrimination studies in which animals are trained to discriminate between amphetamine, MDMA, and placebo, have shown that these drugs are readily discriminable to animals (Goodwin and Baker 2000). Further, when classical hallucinogens (LSD and DMT) are tested in animals trained in this three-way discrimination procedure, only partial substitution for MDMA is observed. Most recently, Tancer and Johanson (CPDD 2001) have conducted drug discrimination studies in humans trained to discriminate between placebo, amphetamine, and mCPP (a serotonin agonist). When MDMA is tested in these people, mixed results are again obtained. Some participants respond to MDMA as if it is amphetamine, while others respond as if it is mCPP. These results further illustrate the mixed actions of MDMA.

The widespread abuse of MDMA with case reports of lethal consequences at the so-called "raves," in conjunction with laboratory evidence for neurotoxic effects has received widespread media attention and alarmed the general public (McCann *et al.* 2000; Ricaurte *et al.* 2000). Any potential beneficial effects MDMA and related drugs may have must be weighed against their possible permanent neurotoxic effects. Similarly, in the 1960s, LSD and other classical hallucinogens were widely used by young people, many of whom were ill prepared for the psychological effects these drugs produce, often with adverse consequences. It is important to distinguish this kind of drug abuse from the use of psychoactive substances within the context of a religious ceremony. As has been shown many times in laboratory studies, set and setting are extremely important determinants of whether a drug experience results in a spiritual epiphany or a "bad trip." As is the case when considering the value of psychoactive drugs for therapeutic purposes, one must look at the risk benefit ratio. This should also be done when considering the use of psychoactive drugs for their effects on spirituality.

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## **HISTORICAL AND CROSS-CULTURAL PERSPECTIVES ON THE USE OF HALLUCINOGENS IN SPIRITUAL PRACTICE**

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The author, a medical anthropologist, examines the use of hallucinogenic substances derived from plants as facilitators of religious ecstasy and to permit individuals to come into first-hand contact with spirit or divinity. These substances are seen to be psychotechnologies to allow tribal elders to manage altered states of consciousness of adolescents and to utilize the properties of the plant hallucinogens as agents of deconditioning of youth and to heighten religious experiences deemed important for social survival. There is a movement historically from exoteric rituals of hallucinogenic drug use, open and accessible to all adolescents and adults, to esoteric rituals, shrouded in secrecy and limited to elite groups of society, much like the Eleusinian mysteries described in ancient Greece. These patterns are illustrated by several cultural examples: the Australian Aborigines' use of pituri in ritual context (*Duboisia* spp); the Fang of Equatorial African and the use of *Tabernanthe iboga*; the ancient Maya and the ritual use of *Nymphae ampla*; the Brazilian church, *Unaoi do Vegetal* use of *ayahuasca* (*Banisteriopsis caapi*).

Findings show that access to supernatural power and the unitive experience were highly valued among hunter/gatherers, incipient and intensive agriculturists, and in pristine state societies of antiquity. Little, if any, abuse potential was found. Most of the plants in traditional societies were of limited availability, were given in religious ritual settings in natural environments with all the senses engaged, with elders and religious leaders present to ensure a smooth interior voyage and were laden with educational and didactic contact to reassure the individual. In traditional societies of the world, stereotypic visions were eagerly sought after to indicate that contact with the realm of the sacred had occurred.

Drug tourism throughout the Third World is a growing phenomenon in response to the demands of Western individuals seeking drug experiences abroad. Knowledgeable men and women are toured in small groups by Western tour guides to distant exotic places where they participate in drug rituals among so-called native shamans or witchdoctors. This fits the category of what has been called charlatan psychiatry, a long tradition for example, in Latin America, of non-authentic folk healers with malicious and fraudulent intention who provide hallucinogenic plant drugs in ritual settings for their personal gain. Unscrupulous practitioners exploit their victims and are conscious of the farce in which they are involved. The hallucinogenic plants in question have never been used traditionally in the way that the self-styled healers use them and there are numerous psychological casualties. The drug tourism is shrouded in special rhetoric and travel literature includes terms like "advanced shamanic training." The drug tourist is desperate to find the vanishing primitive. The westerner is not involved in a native ritual of spiritual dimensions as he has been led to expect but rather in a staged drama to turn him on and to extract his cash. There is an evil exploitative aspect of this drug tourism that is impossible to ignore. These so-called native healers are common drug dealers dressed for deception. They provide the exotic setting and prepare the tourist to have an authentic personal experience.

Throughout history, societies recognized that the power of the mind-altering plants was acknowledged to belong to special realms constrained with taboos and rituals, and a pathway to the spiritual. Anyone who entered those portals had to be properly prepared for the journey.

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## CONTEMPORARY PERSPECTIVES ON THE USE OF HALLUCINOGENS IN SPIRITUAL PRACTICES

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Hallucinogenic plants and chemicals can sometimes give access to an unusual set of mental states, variously called transcendent, mystical, or unitive. The term "entheogen" (literally, "giving rise to divinity within") has been coined to emphasize this special property, which appears to act most frequently under conditions ("set and setting") designed for that purpose.

Numerous cultures throughout history have made religious use of entheogenic plants, giving rise to traditions from which some contemporary entheogen uses follow directly. Living examples include the use of peyote in the Native American Church, the religious uses of ayahuasca in South America, and the ritual use of psilocybin mushrooms in Mesoamerica. Other contemporary uses have resulted from individuals and groups incorporating entheogens into religious traditions that do not otherwise use them. Walter Pahnke's 1962 Good Friday Experiment in a Christian chapel stands as the most thoroughly documented and measured instance of the capacity of certain psychoactives to greatly increase the probability of profound religious experience.

Probably most contemporary entheogen use takes place with no explicit religious or ritual context, though distinctly religious experiences have sometimes occurred, nonetheless, as illustrated by writer Aldous Huxley, religion scholar Huston Smith, and Alcoholics Anonymous founder Bill W.

The modern psychology of religion has characterized religious experience in terms such as unity (direct perception of connectedness or oneness), transcendence of time and space, and sense of sacredness, with emotions ranging from awe (fascination and terror) to deeply felt positive mood. Valid and reliable psychological instruments are now available to measure these phenomena.

Entheogen experiences, in and out of formal religious contexts, are sometimes credited with providing profound insights. Huxley's claim is not atypical in its enthusiasm: "The mescaline experience is, without any question, the most extraordinary and significant experience available to human beings this side of the Beatific Vision. To be shaken out of the ruts of ordinary perception, to be shown for a few timeless hours the outer and inner worlds, not as they appear to an animal obsessed with survival or to a human being obsessed with words and notions, but as they are apprehended, directly and unconditionally, by Mind at Large – this is an experience of inestimable value to anyone." In religious language, such experiences are epiphanies, theophanies, moments of illumination, states of grace, and so forth.

Acting in combination with other individual and social factors, such episodes have long been linked to positive, and sometimes enduring, changes in emotional well-being, beliefs, values, and behaviors. Knowledge of these factors has been accumulated anecdotally, but the area has not much been studied scientifically. The immediate effects of an entheogen and the long-term consequences of the experience are likely to vary with characteristics of the



ingestor, preparation and intentions of the ingestor, the substance and dose, and the social surround. The social surround includes the behavior of formal or informal "guides," the existence and nature of rituals and communities of seekers that help contain the experience and form its meaning, ongoing contact with guide(s) and community, and the nature and intensity of pressures from the wider society.

Were it not for the legal classification of most entheogens as Schedule I drugs, it would go without saying that the examples of entheogen use given earlier bear virtually no resemblance to the patterns of abuse and addiction frequently seen with drugs such as alcohol, cocaine, and heroin. Entheogens do present characteristic risks, including unwise drug-induced behavior and (rarely) lasting psychological distress. Virtually all of the documented cases of harm have come from haphazard use, rather than entheogenic ceremonies such as those of the Native American Church.

More subtle harms can follow when entheogens are put to less than their "highest and best use" (a term borrowed from land use planning). For the individual, lesser uses carry an opportunity cost, insofar as the same investment of time and substance could have yielded greater outcomes. For the community, trivialization of the substance, its use, or the consequent experiences lowers collective expectations and hence may work to the detriment of future use outcomes. Opposite to trivialization, it is also possible to expect too much of the entheogen itself. In religious language, these are, respectively, the profaning of a sacrament and idolatry. In addition, profound religious experiences themselves carry risks of inducing grandiosity or, in religious terms, spiritual pride.

For some 35 years, there has been a near-embargo on scientific research using these substances in humans. A few studies are now being mounted, though the aim of most is to investigate possible applications of the substances to treat mental disease. Thus, an opportunity remains: to study in healthy volunteers the states of consciousness that entheogens can bring about, the determinants of those states, and their short- and long-term consequences, individual and social.

Walter Houston Clark offered in his book *The Psychology of Religion* (1958) a framework for appraising religion. Its first key feature is the inner experience of an individual when he or she senses a "beyond" – what is taken to be a more fundamental aspect of the universe than is ordinarily sensed. The second feature is the effect of such experience on behaviors as one actively attempts to live one's life in accord with values derived from that inner experience. The application of Clark's framework to the use of entheogens constitutes a research program of significant potential fertility.

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## APPLICATION OF SCIENTIFIC METHODS TO THE STUDY OF SPIRITUAL EXPERIENCE

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The study of spiritual experience has until recently been the twice removed step child of psychology. Among the founders of psychology, especially in America, it was widely assumed that religious experiences could be reductively explained by neurophysiological processes. Psychological explanations were offered as reductive interpretations of religious phenomena, including not only transcendent experiences but persistently related experiences such as supposed paranormal and spiritual experiences (Coon 1992; Hood 2000). Interest in religion and religious experience rapidly declined in the face of the assumption that psychology had adequately explained most religious phenomena of interest, many which were declared to be pathological.

A re-emergence of interest in the psychology of religion beginning in the 1960s can be partly attributed to the influence of a few investigators who insisted that the proper description of religious phenomena defied purely naturalistic or neurophysiological explanations. Never doubting that neurophysiology is involved in experience, the investigation of religious experience suggested that reductive explanations were insufficient and that more sophisticated scientific studies of religious experience were needed. The necessary advance came when investigators began not simply to apply measurement paradigms to the psychology of religion, but to derive and focus their measurements with phenomenological and other theoretical perspectives (Gorsuch 1984).

The contemporary psychology of religion has developed reliable and valid measures of religious phenomena on par with constructs in any mainstream area of modern psychology (Hill and Hood 1999). It is widely accepted that religious experience is neither necessarily pathological nor inherently immune to scientific study. While correlational methods dominate the field, experimental and quasi-experimental methods are not uncommon (Hood *et al.* 1996). Among the religiously devout, the report of religious experience is associated with an intrinsic religious orientation; among the non-religious, similar experiences occur but without a religious interpretation. Generally the report of religious experience is related to openness to experience, and to a variety of indices of well-being. Religious experience can be facilitated by a variety of practices and situations, including prayer or meditation, solitude, and entheogens (Hood 1995). Set and setting factors are especially relevant in determining whether or not experiences are given a religious interpretation.

While the psychology of religion is still fairly described as mainstream psychology's stepchild, recent research in psychology has shown a demonstrative movement to focus upon the study of spirituality as opposed to religion making spirituality a stepchild of the modern scientific study of the psychology of religion (Pargament 1999). The well-documented emergence of a significant group of persons who identify themselves as "spiritual but not religious" has turned attention to the nature of experiences that often have been interpreted or even reified in religious traditions but need not be. While those who identify themselves as "equally spiritual and religious" can be documented to have spiritual and religious experiences for which their religious beliefs provided them adequate expression and even explanation, those who reject religion can be shown to have similar experiences for which they reject the religious interpretations. Thus, a dominant paradigm in the study of religious and spiritual experience assumes that experience can be distinguished from interpretation, allowing for a common core of identical experiences to be differentially interpreted. Among those who support this common-core position are researchers who have sought to identify a limited range of experiences that can be scientifically identified as uniquely spiritual, but not necessarily religious. Among the major contenders are mysticism (typically identified as an experience of union) and perhaps more generally, transcendence (in which the individual directly experiences an interconnectedness with all of reality). Both mysticism and transcendence are best understood as multidimensional constructs and have been reliably measured as such (MacDonald *et al.* 1999a,b). This approach allows for experience to be separated from interpretation, implying that, whatever the role of language and culture in shaping experience, there is enough commonality in the underlying determinants of mystical and transcendent experience to

suggest both a universal neurophysiological basis for such experiences. Perhaps more controversially, it suggests an ontological basis that is transcendent to a purely neurophysiological explanation (Austin 1999, d'Aquil and Newberg 1999).

Thus, whether the psychology of religious experience or spirituality is the focus, the application of scientific methods has shown that the experiences can be both measured and scientifically manipulated. Whatever their final explanation might be, they are a proper focus of scientific investigation. That suggests that these experiences are not forever fated to be stepchildren but shall find their proper home. If this home is not in psychology, it surely will be under a multidisciplinary canopy of investigators whose common commitment is to the rigorous application of scientific methods to reality not simply as it is, but as it appears to be for those who have these experiences.

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## HALLUCINOGENS AND RELIGION: HISTORICAL TO SCIENTIFIC PERSPECTIVES – DISCUSSANT

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Research into hallucinogens was prematurely terminated in the 1960s, when some of the drugs became street drugs of abuse. Research into these agents can be described as high risk with potential high gain. While these agents can produce substantial behavioral toxicity, they also have the potential to lead to useful insights, positive behavioral change, and increased knowledge of the brain. Research could include studies on the nature of the hallucinogen-induced experience itself, the role of set and setting, behavioral changes that ensue and the neuroscience aspects involved, e.g. neuroendocrine and imaging studies. The key question from a policy point of view is: How should the conflict between the country's compelling need not to interfere with religious practice and the equally compelling need to protect our people, especially the vulnerable young, be resolved? This is a difficult and cloudy issue. However, the current position that permits bonafide members of the Native American Church to use peyote in a controlled communitarian setting may serve as a useful model and could be expanded. The use by individuals in a non-communitarian setting creates too much risk at this time as does permitting use by pseudo-religious groups whose only purpose is the drug use.

## BIOLOGICAL EVALUATION OF COMPOUNDS FOR THEIR PHYSICAL DEPENDENCE POTENTIAL AND ABUSE LIABILITY. XXV. DRUG EVALUATION COMMITTEE OF THE COLLEGE ON PROBLEMS OF DRUG DEPENDENCE (2001)

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### THE DRUG EVALUATION COMMITTEE (DEC) AND ITS MEMBERSHIP

Dr. A. Coop replaced Dr. A. E. Jacobson as the sole Biological Coordinator of DEC, CPDD in 2000. Dr. Jacobson and Dr. Coop worked closely together during 1999 and 2000, and Dr. Jacobson is to be congratulated on his efforts to ensure a smooth transition. Dr. Coop (UMB) is the fourth DEC Biological Coordinator (the initial two were Drs. N. Eddy and E. May). The other members of DEC remained unchanged this year; they are in DEC's two analgesic testing groups, at Virginia Commonwealth University (VCU, Drs. L. Harris, M. Aceto, P. Beardsley) and the University of Michigan (UM, J. Woods (DEC Chair), J. Traynor), and three stimulant/depressant testing groups, at the University of Mississippi (UMS, W. Woolverton), University of Texas Health Science Center San Antonio (UTHSCSA, C. France), and UM (G. Winger, J. Woods). Drs. T Cicero and A. E. Jacobson act as emeritus members. The DEC reports to the CPDD's Liaison Committee for Drug Testing and Evaluation (N. Ator, Chair). Members of both that CPDD Committee and the Industry Relations Committee (W. Schmidt, Chair), as well as NIDA, attend DEC's meeting held during the Annual Scientific Meeting of the CPDD. One or two other DEC meetings are held during the year to discuss the work which has been accomplished and future plans. Separate meetings are held at VCU quarterly with the members of the VCU Analgesic Testing Group, as well as Drs. E. May and E. Bowman, the DEC Biological Coordinator, and a NIDA representative, to discuss the results obtained from the VCU testing and research program.

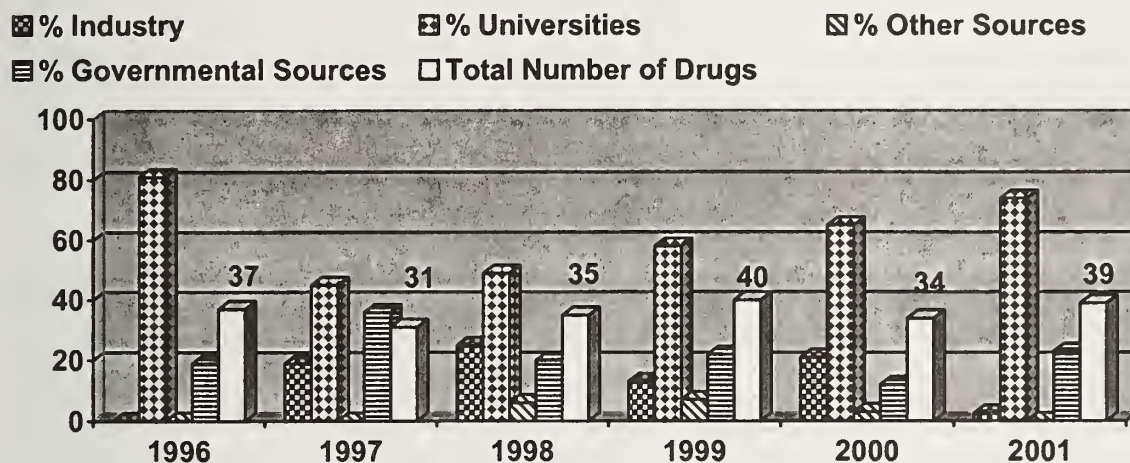
Data were released for publication this year on 39 different compounds evaluated by DEC's Analgesic Testing Program (Figure 1). Of these, 33 compounds were evaluated at VCU (antinociceptive assays in mice - tail flick, hot-plate, and phenylquinone, and the tail-flick antagonist assay, as well as substitution for morphine and precipitated withdrawal assays in rhesus monkeys), and 33 at UM (binding affinity to the  $\mu$ ,  $\delta$ , and  $\kappa$  opioid receptors, and GTP $\gamma$ S functional studies). The compounds came from two main sources: 74% from universities and 23% from governmental sources. The remaining compounds (3%) came from pharmaceutical industry, a figure lower than previous years. One compound was released for publication this year by the groups in the Stimulant/Depressant Testing Program.

Joint publications based on the data gathered under DEC auspices from VCU, UM, UMB, and NIH, have been published (Coop *et al.* 2000, May *et al.* 2000a, May *et al.* 2000b). In addition, a poster based on the data gathered by DEC was presented at the 63<sup>rd</sup> Annual Meeting of the College on Problems of Drug Dependence (Coop *et al.* 2001). A DEC report on CPDD 0056, a sulfur containing derivative of amphetamine, was communicated to the World Health Organization.

### EXPERIMENTAL OBSERVATIONS

The names of the compounds that were released for publication this year are listed in Table 1, and their molecular structures and a summary of their *in vivo* and *in vitro* data are in Tables 2 to 9. Similar to previous years (Coop and Jacobson, 2001), the examined compounds are classified according to their molecular structure: 4,5-epoxymorphinans in Tables 2 and 3; 3-O-substituted 4,5-epoxymorphinans in Table 4; miscellaneous opioids in Table 5; 6,7-benzomorphans in Table 6; miscellaneous compounds (those which do not fall into the usual opioid classes) are listed in Tables 7 and 8. Compounds evaluated by the Stimulant/Depressant testing groups are shown in Table 9. The more interesting compounds evaluated during the year are discussed below. For compounds that have been previously evaluated, the new data are discussed in relation to the published data.

**FIGURE 1. DEC ANALGESIC PROGRAM: PERCENT, TOTAL NUMBER, AND SOURCE OF EXAMINED DRUGS (1996-2001)**



NIH 10497 (Table 2) possesses an unusual *N*-1*R*-1-cyclopropylethyl substituent, similar to the  $\mu$ -antagonist conferring *N*-cyclopropylmethyl. Previous reports (1989, 2001) showed that NIH 10497 is not active as a morphine antagonist in the mouse, and can completely substitute for morphine in monkeys, indicating a  $\mu$ -agonist profile. Side-effects seen in the monkeys (e.g. salivation) also suggested  $\kappa$ -agonist activity, consistent with the high affinity seen for both  $\mu$  and  $\kappa$  receptors. However, in contrast, GTP $\gamma$ S functional data show that NIH 10497 has low  $\mu$  efficacy, which is not consistent with the *in vivo* substitution data. This is consistent with the fact that NIH 10497 appears to be relatively free of  $\mu$ -opioid dependence liability in the rat. Table 2 shows that opioid subtype testing in the tail-flick assay demonstrates predominantly  $\kappa$  agonist effects. It thus appears that the activity of NIH 10497 is due to a profile of  $\kappa$  agonism.

NIH 10978 in Table 2, is an analog of the  $\delta$ -opioid selective antagonist naltrindole (NIH 10990, Table 2) possessing a 2-methylallyl *N*-substituent. The introduction of this *N*-substituent gives rise to an extremely  $\delta$  selective ligand in binding assays, and  $\delta$ -antagonism in GTP $\gamma$ S functional assays. These data suggest that a 2-methylallyl substituent may be superior to the traditional cyclopropylmethyl group in morphinan-based  $\delta$  selective ligands. In contrast, NIH 10979 (Table 2), with an unusual *N*-cyclohexylethyl substituent, actually displays preference for  $\mu$  receptors over  $\delta$ . This demonstrates that the nature of the *N*-substituent is an extremely important factor in the binding profile of analogs of naltrindole (Coop *et al.* 2000; McLamore *et al.* 2001).

NIH 10986 and NIH 10989 (Table 3) are both well known  $\mu$  antagonists - their activity in GTP $\gamma$ S assays was consistent with this profile. NIH 10985 and NIH 10990 are both considered  $\delta$  selective antagonists but, as can be seen from Table 3, they both display potent  $\mu$  antagonism (only 4-5 fold less potent than naltrexone). These data are consistent with the previous findings that NIH 10990 effectively exacerbates withdrawal in the morphine dependent monkey. NIH 10987 (buprenorphine) (Table 3) is a potent  $\mu$  receptor mediated analgesic which is employed clinically (Lewis, 1985). Its partial  $\mu$  agonist activity can be seen *in vitro*, in that it efficiently acts as an antagonist of the  $\mu$  agonist DAMGO, but only reverses 67% of the response of DAMGO.

Table 4 contains three compounds which do not have the 3-phenolic substituent usually required for high potency at the opioid system. The cinnamoyl ester of NIH 11037 will hydrolyze rapidly *in vivo* to give naltrexone, and indeed was shown to be a potent  $\mu$  and  $\kappa$  antagonist *in vivo*. NIH 11028 contains a 3-methyl ether which undergoes metabolism more slowly to naltrexone, and was shown to possess lower morphine antagonist potency than NIH 11037 in the mouse. NIH 11015 (Table 4) displayed the expected  $\mu$  agonism, and is probably O-demethylated to the more active phenol *in vivo*.

**NIH 10945** (Table 5) possesses a benzomorphan-like structure, but with the basic nitrogen in a unique position. It displays high affinity for  $\mu$  and  $\kappa$  receptors, yet has only feeble opioid activity *in vivo*. It is possible that NIH 10945 has problems with transport into the CNS, or is conjugated rapidly on the nitrogen to an inactive species. **NIH 11026** (Table 5) is the unnatural enantiomer of the minor opium alkaloid oripavine. The natural isomer is known to be an antinociceptive agent (Gomez-Serranillos *et al.* 1998), but interest in the unnatural enantiomers was kindled after the finding that (+)-thebaine (the 3-O-methyl ether of (+)-oripavine) also displayed opioid mediated antinociceptive properties (Aceto *et al.* 1999). As 3-phenols in the natural series tend to possess greater potency than the 3-methyl ethers, it was of interest to investigate if similar SAR was present for the unnatural isomers. As can be seen from Table 5, NIH 11026 showed only weak opioid agonist activity. This suggests that the unnatural thebaines may possess a very different SAR to the natural isomers.

A series of benzomorphans is shown in Tables 6a and 6b. It has been previously reported that *N*-benzyl substituted benzomorphans display poor *in vivo* and *in vitro* opioid activity (Coop and Jacobson, 2001), and **NIH 10994**, **NIH 10995**, **NIH 11003**, **NIH 11004**, and **NIH 11021** follow the same trend. **NIH 11020** (Table 6a) however, has an unusual profile. NIH 11020 possesses high affinity for  $\mu$  and  $\kappa$  receptors, yet is completely inactive *in vivo* as an antinociceptive agent or as a morphine antagonist. The reason for this profile is not obvious, but underscores the need for *in vivo* assays together with *in vitro* assays. **NIH 11013** and **NIH 11014** are the (-) and (+) enantiomers of a benzomorphan with an *N*-phenylpropyl substituent. As expected, NIH 11013 displays higher affinity for opioid receptors, and greater potency in most *in vivo* assays, yet the (+)-isomer NIH 11014 is of greater potency in the antiwrithing assay (PPQ). Indeed, NIH 11014 is unusually active for a (+)-isomer in all assays. Interestingly, side-effects noted in the monkey assays tend to suggest that the agonism may be mediated through  $\kappa$  receptors. **NIH 11006** (Table 6a) possesses a cyclobutylmethyl *N*-substituent - a substituent generally regarded as being associated with  $\mu$  antagonism, and the GTP $\gamma$ S assays show low  $\mu$  efficacy. The side effects noted in the monkey (e.g. salivation) tend to suggest a strong  $\kappa$ -opioid component to the antinociceptive activity of NIH 11006. **NIH 11023** (Table 6a), with a 3-methylbutyl *N*-substituent, was about ten-fold less potent than NIH 11006 in the rodent antinociceptive assays, but side-effects again suggest a strong  $\kappa$ -mediated component from the monkey assays.

The notorious  $\gamma$ -hydroxybutyrate (GHB, **NIH 10947**) (Table 7) has been widely studied by DEC. It has previously been reported (Coop and Jacobson, 2001) that NIH 10947 has little opioid-like activity alone, but acts synergistically with morphine in PPQ. In withdrawn morphine-dependent monkeys, NIH 10947 attenuated withdrawal at low doses, but exacerbated withdrawal at higher doses. In addition, when NIH 10947 was given with morphine to morphine tolerant mice, antinociception was partially restored (Aceto and Bowman, 2000). These data suggested potential therapeutic uses for GHB in the treatment of pain in morphine tolerant patients, and potential safety issues for opioid abusers if they also administer GHB. It has now been shown that synergism may occur between NIH 10947 and ethanol - an extremely important finding as NIH 10947 is often placed into alcoholic drinks. Indeed, in combination with ethanol, mice became ataxic at relatively low doses compared to doses required of GHB alone. The interaction of GHB with the opioid system, led to the study of the effects of the putative GHB antagonist **NIH 11016** (NCS-382 Maitre *et al.* 1990) (Table 7) on the opioid system. With the exception of feeble, non-dose related attenuation of withdrawal in the monkey, no effects were seen. These data indicate that NCS-382 can be employed as a GHB antagonist when investigating the effects of GHB on the opioid system, without the antagonist having direct effects on the opioid system itself.

**NIH 11018** ((-)-nicotine, Table 8) demonstrated antinociception in mice and appeared to exacerbate withdrawal signs in the monkey. Convulsions were noted in all rodent assays, and the apparent exacerbation of withdrawal (increased retching and vocalization were noted) are probably due to the stimulant effects of nicotine. The (+)-isomer of nicotine (**NIH 11017**) showed lower potency as an antinociceptive agent, and a lower incidence of CNS effects. Agmatine (**NIH 11035** Table 8) has been reported to attenuate morphine withdrawal signs in rats (Reis and Regumathan, 2000). Consistent with this report, it is shown in Table 8 that a suggestion of attenuation of withdrawal is seen in the monkey, but is not significant at 6 mg/kg.

Racemic mecamylamine **CPDD 0059** (Table 9) was previously reported by the analgesic group as NIH 11010 (Coop and Jacobson, 2001). As with the resolved enantiomers (CPDD 0057 and CPDD 0058), no reinforcing effects were observed in methohexital trained monkeys.

**TABLE 1. EVALUATED COMPOUNDS**

NIH#	COMPOUND NAME	TABLE #- Evaluator
10497	<i>N</i> -(1 <i>R</i> -1-Cyclopropyl)ethylnormorphine hydrochloride	2-VCU
10945	(+/-)-(5 <i>S</i> ,8 <i>S</i> ,9 <i>R</i> )-8-Amino-3-hydroxy-5,9-methano-9-(methoxymethyl)-5-methylbenzocyclooctene	5-VCU/UM
10947	$\gamma$ -Hydroxybutyric Acid, sodium salt	7-VCU
10978	<i>N</i> -(3-Methylallyl)noroxymorphindole	2-VCU/UM
10979	<i>N</i> -Cyclohexylethylnoroxymorphindole.HCl	2-VCU/UM
10985	7-Benzylidene-7-dehydronaltrexone.HCl (BNTX)	3-UM
10986	Naltrexone.HCl	3-UM
10987	Buprenorphine.HCl	3-UM
10988	Norbinaltorphimine.HCl (norBNI)	3-UM
10989	14 $\beta$ -( <i>p</i> -Chlorocinnamoylamino)-7,8-dihydro- <i>N</i> -cyclopropylmethylnormorphinone mesylate (Clocinnamox)	3-UM
10990	Naltrindole.HCl	2-UM
10992	(+)-(1 <i>S</i> ,5 <i>S</i> ,9 <i>S</i> )-2-Acetamido-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan	6-VCU/UM
10994	(-)-(1 <i>R</i> ,5 <i>R</i> ,9 <i>R</i> )-5,9-Dimethyl-2'-hydroxy-2-(4-trifluoromethylbenzyl)-6,7-benzomorphan .oxalate	6-VCU/UM
10995	(+)-(1 <i>S</i> ,5 <i>S</i> ,9 <i>S</i> )-5,9-Dimethyl-2'-hydroxy-2-(4-trifluoromethylbenzyl)-6,7-benzomorphan .oxalate	6-VCU/UM
11003	(+)-(1 <i>S</i> ,5 <i>S</i> ,9 <i>S</i> )-5,9-Dimethyl-2'-hydroxy-2-(2-trifluoromethylbenzyl)-6,7-benzomorphan . HCl	6-VCU/UM
11004	(-)-(1 <i>R</i> ,5 <i>R</i> ,9 <i>R</i> )-5,9-Dimethyl-2'-hydroxy-2-(2-trifluoromethylbenzyl)-6,7-benzomorphan . HCl	6-VCU/UM
11005	4-(3-hydroxyphenyl)-4-(1-oxo-propyl)-1-(2-trifluoromethylbenzyl)piperidine.HCl	5-VCU/UM
11006	(-)-(1 <i>R</i> ,5 <i>R</i> ,9 <i>R</i> )-2-Cyclobutylmethyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan. HCl	6-VCU/UM
11007	(+)-(1 <i>S</i> ,5 <i>S</i> ,9 <i>S</i> )-2-Cyclobutylmethyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan. HCl	6-VCU/UM
11011	(+)-(1 <i>S</i> ,5 <i>S</i> ,9 <i>S</i> )-2-Cyclohexylmethyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan. HCl	6-VCU/UM
11012	(-)-(1 <i>R</i> ,5 <i>R</i> ,9 <i>R</i> )-2-Cyclohexylmethyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan. HCl	6-VCU/UM
11013	(-)-(1 <i>R</i> ,5 <i>R</i> ,9 <i>R</i> )-2-(3-Phenylpropyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan. HCl	6-VCU/UM
11014	(+)-(1 <i>S</i> ,5 <i>S</i> ,9 <i>S</i> )-2-(3-Phenylpropyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan. HCl	6-VCU/UM
11015	Thevinone.oxalate	4-VCU/UM
11016	NCS-382, sodium salt	7-VCU/UM
11017	( <i>R</i> )-(+)-Nicotine di- <i>d</i> -tartrate	8-VCU/UM

11018	(S)-(-)-Nicotine di- <i>l</i> -tartrate	8-VCU/UM
11019	Caffeine tartrate	8-VCU
11020	(-)-(1 <i>R</i> ,5 <i>R</i> ,9 <i>R</i> )-5,9-dimethyl-2-(3-fluorobenzyl)-2'-hydroxy-6,7-benzomorphan.oxalate	6-VCU/UM
11021	(+)-(1 <i>S</i> ,5 <i>S</i> ,9 <i>S</i> )-5,9-dimethyl-2-(3-fluorobenzyl)-2'-hydroxy-6,7-benzomorphan.oxalate	6-VCU/UM
11022	(+)-(1 <i>S</i> ,5 <i>S</i> ,9 <i>S</i> )-2-(3-Methylbutyl)- 5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.oxalate	6-VCU/UM
11023	(-)-(1 <i>R</i> ,5 <i>R</i> ,9 <i>R</i> )-2-(3-Methylbutyl)- 5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.oxalate	6-VCU/UM
11024	Metanicotine.oxalate	8-VCU
11025	2-(2-phenethyl)-1,2,3,4-tetrahydroisoquinoline.oxalate	8-VCU/UM
11026	(+)-Oripavine.oxalate	5-VCU/UM
11028	3-O-Methylnaltrexone.HCl	4-VCU/UM
11034	L-Lobeline	8-VCU
11035	Agmatine.sulfate	8-VCU
11037	3-O-Cinnamoylnaltrexone.HCl	4-VCU/UM
CPDD 0059	(+/-)-Mecamylamine.HCl	9-S/D Group

#### NOTES FOR TABLES 2 - 9

Rounded numbers are used; precise values and details of the procedures are given in the VCU and UM reports (Aceto *et al.* 2002, Woods *et al.* 2002). "Inactive" is stated when an ED<sub>50</sub> or AD<sub>50</sub> is not reached. HP = hot plate assay; PPQ = phenylquinone antiwrithing assay; TF = tail flick assay; NTI = naltrindole (delta antagonist); norBNI = norbinaltorphimine (kappa antagonist); β-FNA = β-funaltrexamine (mu antagonist).

##### 1) Antinociceptive reference data:

Morphine ED<sub>50</sub> (mg/kg): Hot Plate = 0.8; Phenylquinone = 0.23; Tail-Flick = 5.8; Tail-Flick Antagonism vs. morphine (naltrexone AD<sub>50</sub> = 0.007; naloxone AD<sub>50</sub> = 0.035).

##### 2) *In Vitro*:

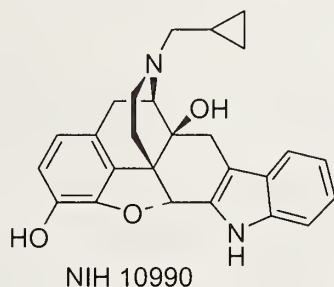
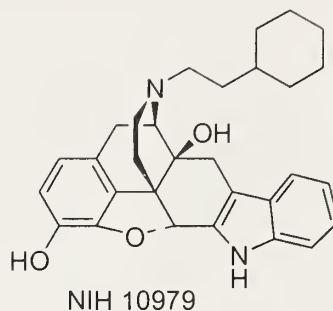
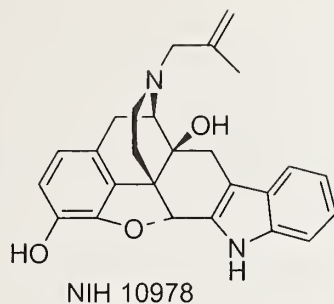
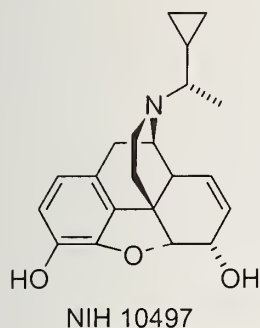
Subtype selective binding affinity using recombinant receptors: μ (C<sub>6</sub> rat glioma cells expressing rat μ receptor), κ (CHO cells expressing human κ receptor), and δ (C<sub>6</sub> rat glioma cells expressing rat δ receptor). Affinity was assessed through the displacement of [<sup>3</sup>H]-Diprenorphine. K<sub>i</sub> values for standard ligands: μ (DAMGO 7.6 nM, morphine 11.2 nM); δ (SNC80 0.8 nM); κ (U69593 0.3 nM)

[<sup>35</sup>S]GTPγS functional data were obtained employing recombinant receptors as described above. Values are given as EC<sub>50</sub> with % stimulation compared to the standard full agonist (DAMGO, SNC80, U69,593), or the maximum stimulation achieved. μ (ED<sub>50</sub>) morphine = 65 nM (100% stimulation), DAMGO = 34 nM (100% stimulation); δ (ED<sub>50</sub>) SNC80 = 9 nM (100% stimulation), DPDPE = 8.3 nM (60% stimulation); κ (ED<sub>50</sub>) U69,593 = 31 nM (100% stimulation), bremazocine = 0.5 nM (86% stimulation).

References to previous Drug Evaluation Committee annual reports refer to the year of publication.



TABLE 2. 4,5-EPOXYMORPHINANS



ANTINOCICEPTIVE/ANTAGONIST ASSAYS  
(MOUSE ED<sub>50</sub>/AD<sub>50</sub>, s.c., mg/kg)

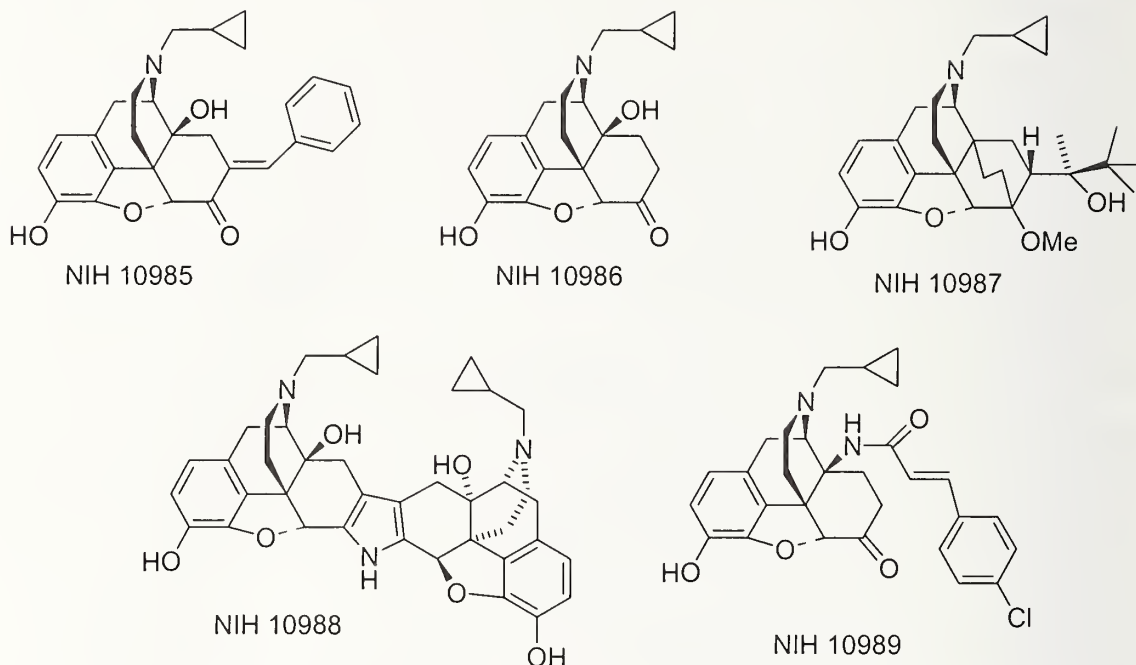
IN VITRO

MONKEY

NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10497 <sup>b</sup>	-	0.03 <sup>a</sup>	2.0 <sup>a</sup>	Inactive <sup>a</sup>	$\mu=0.1, \delta=29, \kappa=1.3^a$	Complete substitution <sup>a</sup>
10978 <sup>c</sup>	Inactive	Inactive	Inactive	Inactive	$\mu=430, \delta=0.68, \kappa=355$	No substitution or exacerbation of withdrawal
10979	2.42	0.39	3.86 <sup>d</sup>	-	$\mu=7.3, ^c \delta=181, \kappa=378$	-
10990 <sup>f,g</sup>	-	-	-	-		

- a) Previously reported (2001). [<sup>35</sup>S]GTP $\gamma$ S assay:  $\mu$  EC<sub>50</sub> = 2191 nM (18.7% stimulation);  $\delta$  EC<sub>50</sub> = 72.2 nM (11.7% stimulation);  $\kappa$  EC<sub>50</sub> = 18.3 nM (78.4% stimulation). Monkey self-administration: maintained rates between saline and codeine; monkey drug discrimination: codeine like; thermal analgesia:  $\mu + \kappa$ , more effective @ 50 than 55 °C; rat primary physical dependence: relatively free of  $\mu$ -opioid dependence liability; naloxone AD<sub>50</sub> (TF): 2.98; vas deferens:  $\kappa$ -profile; rat brain homogenate binding: 2.1 nM.
- b) New data: Opioid subtype testing against ED<sub>80</sub> of NIH 10497 in TF demonstrated weak  $\kappa$ -agonist effects, and no  $\mu$ -agonist effects. Activity of three different samples of NIH 10497 in TF: ED<sub>50</sub> = 4.47 (0.51-39.08); 2.52 (0.67-9.47); 1.67 (0.31-8.96).
- c) [<sup>35</sup>S]GTP $\gamma$ S assay:  $\mu$ : <5% stimulation at 10  $\mu$ M;  $\delta$ : no stimulation at 10  $\mu$ M;  $\kappa$ : no stimulation at 10  $\mu$ M. Antagonism of SNC80 ( $\delta$  agonist) pK<sub>B</sub> = 8.93.
- d) Naloxone AD<sub>50</sub> = 0.1;  $\beta$ -FNA ( $\mu$ g/brain) AD<sub>50</sub> = 1.25; norBNI inactive; NTI inactive.
- e) [<sup>35</sup>S]GTP $\gamma$ S assay:  $\mu$ : EC<sub>50</sub> = 105 nM (52% stimulation).
- f) Previously reported as NIH 10589 (1999, 2000) (See Table 3)
- g) New data: [<sup>35</sup>S]GTP $\gamma$ S assay: AD<sub>50</sub> vs. DAMGO = 7.9 nM.

TABLE 3. 4,5-EPOXYMORPHINANS

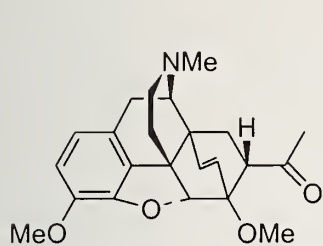


$[^{35}\text{S}]\text{GTP}\gamma\text{S}$  FUNCTIONAL ASSAYS ( $\text{AD}_{50}$  nM  $\pm$  SEM)

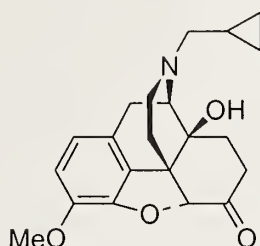
NIH #	Antagonism of DAMGO ( $\mu$ )
10985 <sup>a</sup>	4.1 $\pm$ 1.1
10986 <sup>b</sup>	1.4 $\pm$ 0.3
10987 <sup>c</sup>	1.3 $\pm$ 0.3 (67% reduction of stimulation)
10988 <sup>d</sup>	-
10989 <sup>e</sup>	1.2 $\pm$ 0.2
10990 <sup>f</sup>	7.9 $\pm$ 2.2

- a) Previously reported as NIH 10923 (1998): Inactive in HP, TF, and PPQ. Antagonism of morphine in TF  $\text{AD}_{50}$  = 0.05 mg/kg. Antagonism of : DPDPE in TF  $\text{AD}_{50}$  = 0.04 mg/kg; sufentanyl in TF  $\text{AD}_{50}$  = 4.0 mg/kg; U69,593 in TF inactive.
- b) Previously reported as NIH 8503 (1971) and NIH 9930 (1983, 1984, 1986): Inactive in HP, PPQ, and TF. Antagonism of morphine in TF  $\text{AD}_{50}$  = 0.007 mg/kg. Precipitation of withdrawal in morphine dependent monkeys (potency 10x naloxone)
- c) Previously reported as NIH 8805 (1974) and NIH 10276 (1985, 1986):  $\text{ED}_{50}$  (mg/kg) HP = 0.035, PPQ = 0.016, TF = 0.14. Antagonism of morphine in TF  $\text{AD}_{50}$  = 1.0 mg/kg. Precipitates withdrawal in morphine-dependent monkeys at 0.32 mg/kg. No substitution for morphine observed.
- d) Previously reported as NIH 10588 (1991): Inactive in PPQ, TF, and as an antagonist of morphine in TF. Exacerbated withdrawal in morphine dependent monkeys. Binding against  $[^3\text{H}]\text{-etorphine}$  in rat brain homogenates  $K_i$  = 70 nM; Functional assays (vas deferens) indicated  $\kappa$ -antagonism.
- e) Previously reported as NIH 10443 (1988, 1989, 1990): Inactive in HP, PPQ, and TF. Antagonism of morphine in TF  $\text{AD}_{50}$  = 0.12 (long duration of action). Binding against  $[^3\text{H}]\text{-etorphine}$  in rat brain homogenates  $K_i$  = 0.65 nM; Functional assays (vas deferens) indicated irreversible antagonism. Severe withdrawal in morphine dependent monkeys, which could not be reversed.
- f) Structure in Table 2. Previously reported as NIH 10589 (1990, 2000): Inactive in PPQ, TF, and as an antagonist of morphine in TF. Exacerbates withdrawal in morphine dependent monkeys. Binding ( $K_i$ , nM, monkey brain cortex)  $\mu$  = 9.5,  $\delta$  = 0.21,  $\kappa$  = 20.5.  $pA_2$  vs. DSLET = 9.44,  $pA_2$  vs. sufentanyl = 7.71.

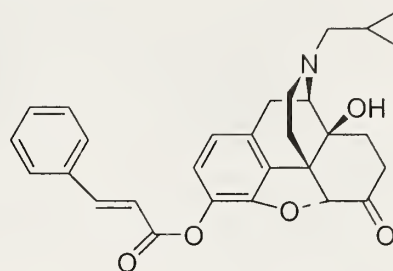
TABLE 4. 3-O-SUBSTITUTED 4,5-EPOXYMORPHINANS



NIH 11015



NIH 11028



NIH 11037

ANTINOCICEPTIVE/ANTAGONIST ASSAYS  
(MOUSE ED<sub>50</sub>/AD<sub>50</sub>, s.c., mg/kg)

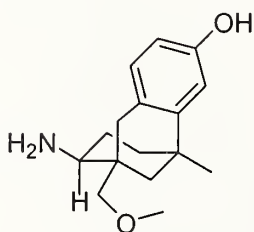
IN VITRO

MONKEY

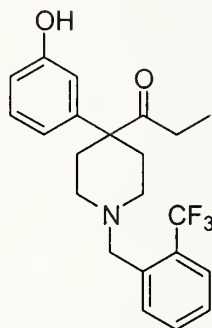
NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
11015 <sup>a</sup>	4.49	2.36	5.56 <sup>b</sup>	-	$\mu=211$ , $\delta=2102$ , $\kappa=6311$	Complete substitution at 2 and 8 mg/kg
11028	Inactive	-	Inactive	0.47 <sup>c</sup>	$\mu=30.8$ , $\delta=589$ , $\kappa=95.2$	-
11037	Inactive	Inactive	Inactive	0.013 <sup>d</sup>	$\mu=18.4$ , $\delta=385$ , $\kappa=30.7$	Precipitated withdrawal at 0.03 and 0.15 mg/kg. <sup>e</sup>

- a) Previously reported as NIH 10631 (1991): ED<sub>50</sub> (mg/kg) - PPQ = 2.0; TF = 8.3. Mouse vas deferens functional assay EC<sub>50</sub> = 1.7 nM (Max. 63% inhibition). Complete suppression of withdrawal signs in withdrawn morphine dependent monkeys.
- b) Naloxone vs. ED<sub>80</sub> NIH 11015 in TF: AD<sub>50</sub> = 0.02.
- c) Antagonism of morphine ED<sub>80</sub> (p.o.) in TF: AD<sub>50</sub> = 2.31. Six hour pretreatment with NIH 11028 - Antagonism of morphine ED<sub>80</sub> (p.o.) in TF: Inactive.
- d) At 30 min. pretreatment. With 4h pretreatment, AD<sub>50</sub> = 2.69. AD<sub>50</sub> vs. ED<sub>80</sub> enadoline = 0.196.
- e) Slightly more potent and longer acting than naloxone.

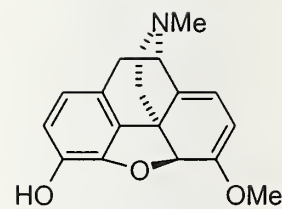
TABLE 5. MISCELLANEOUS OPIOIDS



NIH 10945



NIH 11005



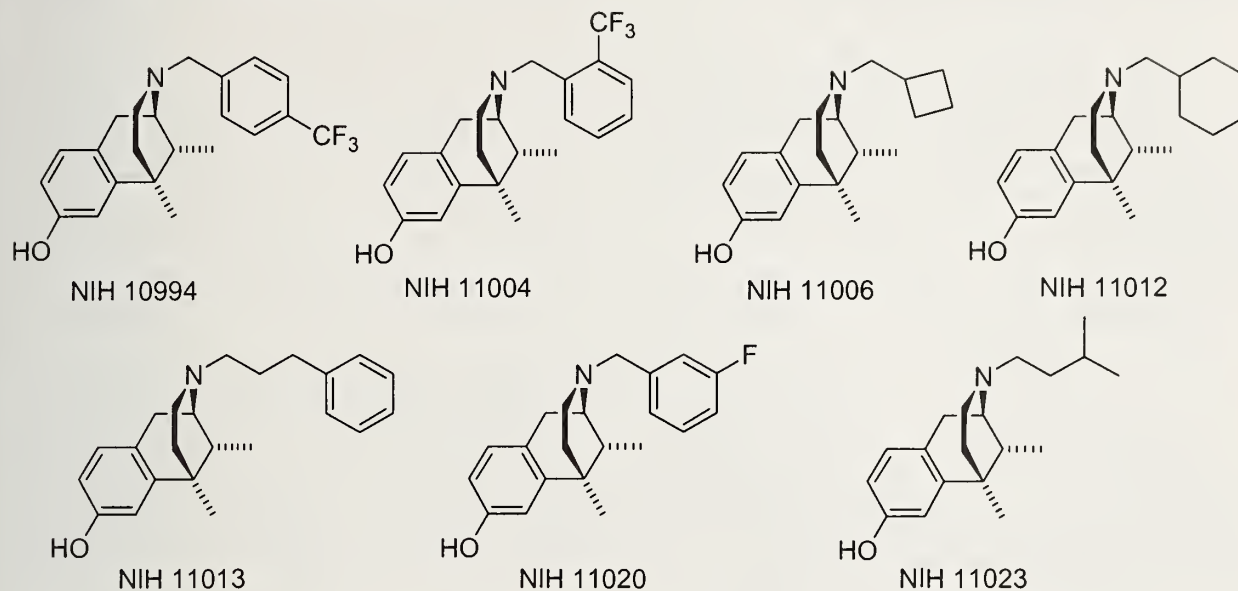
NIH 11026

ANTINOCICEPTIVE/ANTAGONIST ASSAYS      IN VITRO      MONKEY  
(MOUSE ED<sub>50</sub>/AD<sub>50</sub>, s.c., mg/kg)

NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10945	Inactive	3.75 <sup>a</sup>	Inactive	Inactive	$\mu=3.7, \delta=156, \kappa=6.3$	Partial suppression <sup>b</sup>
11005	Inactive	Inactive <sup>c</sup>	Inactive	Inactive	$\mu=546, \delta=119, \kappa=836$	No suppression at 15 mg/kg
11026	Inactive	0.58 <sup>d</sup>	Inactive	Inactive	$\mu=806, \delta=>10,000, \kappa=>10,000$	Partial attenuation of withdrawal signs at 4 and 16 mg/kg

- a) Naloxone AD<sub>50</sub> vs. ED<sub>80</sub> NIH 10945 = 2.63.
- b) Lower dose (4 mg/kg) appeared more effective than higher dose (16 mg/kg).
- c) 49% inhibition at 30; 51% at 60.
- d) Antagonism of ED<sub>80</sub> in PPQ:  $\beta$ -FNA = 46% at 10 $\mu$ g (i.c.v.); norBNI = 60% at 10 mg/kg; NTI inactive.

TABLE 6a. (-)-6,7-BENZOMORPHANS

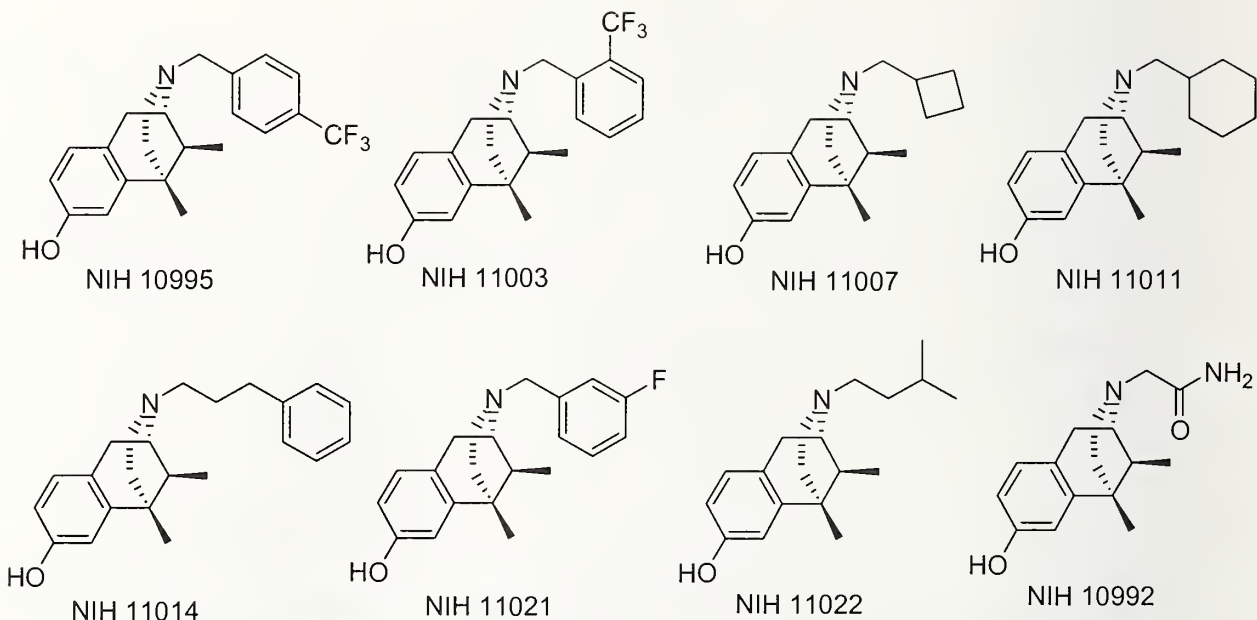


ANTINOCICEPTIVE/ANTAGONIST ASSAYS      IN VITRO      MONKEY  
(MOUSE ED<sub>50</sub>/AD<sub>50</sub>, s.c., mg/kg)

NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10994	Inactive	27.4	Inactive	Inactive	$\mu=1435$ , $\delta>10,000$ , $\kappa>1872$	Neither substituted nor exacerbated withdrawal at 4 and 16 mg/kg
11004	Inactive	Inactive	Inactive	Inactive	$\mu=335$ , $\delta=1091$ $\kappa=149$	Neither substituted nor exacerbated withdrawal at 4 and 16 mg/kg
11006	0.12	0.04	0.26 <sup>a</sup>	-	$\mu=2.9$ , $\delta=11.3$ $\kappa=0.74$ <sup>b</sup>	Complete substitution at 0.25 mg/kg <sup>c</sup>
11012	Inactive	14.62	Inactive	3.7	$\mu=26$ , $\delta=315$ , $\kappa=13$	Neither substituted nor exacerbated withdrawal at 0.75 and 3 mg/kg <sup>d</sup>
11013	9.63	4.59	8.86	-	$\mu=11$ , $\delta=47$ , $\kappa=34$	Partial substitution at 3.5 mg/kg <sup>e</sup>
11020	Inactive	Inactive	Inactive	Inactive	$\mu=16.3$ , $\delta=351$ , $\kappa=7.9$	Neither substituted nor exacerbated withdrawal at 4 and 16 mg/kg
11023	Inactive	0.39	2.5	-	$\mu=16.4$ , $\delta=36.7$ , $\kappa=7.3$	At 2 and 8 mg/kg, reduced withdrawal signs, but did not substitute for morphine <sup>f</sup>

- a) Antagonism of NIH 11006 in TF: Naloxone vs. ED<sub>80</sub> - AD<sub>50</sub> = 0.84; NTI vs. ED<sub>80</sub> 19% at 30; norBNI vs. ED<sub>80</sub> 16% at 30;  $\beta$ -FNA vs. ED<sub>80</sub> - AD<sub>50</sub> = 0.49.
- b) [<sup>35</sup>S]GTP $\gamma$ S assay:  $\mu$  EC<sub>50</sub> = 2.0 nM (25.5% stimulation);  $\delta$  EC<sub>50</sub> = 54.3 nM (22.3% stimulation);  $\kappa$  EC<sub>50</sub> = 3.1 nM (57.9% stimulation).
- c) Other effects included ataxia, jaw sag, salivation, tremor, eyelid ptosis.
- d) Jaw sag was noted at 3 mg/kg, and tremors were noted at 12 mg/kg which prevented assessment.
- e) Other effects included ataxia, jaw sag, eyelid ptosis. Prompt onset of action, and duration of action less than morphine.
- f) Other effects included ataxia, jaw sag, eyelid ptosis.

TABLE 6b. (+)-6,7-BENZOMORPHANS

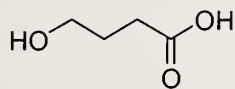


ANTINOCICEPTIVE/ANTAGONIST ASSAYS IN VITRO MONKEY  
(MOUSE ED<sub>50</sub>/AD<sub>50</sub>, s.c., mg/kg)

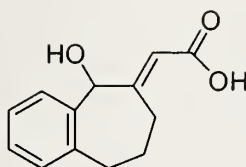
NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10992	Inactive	4.05	Inactive	Inactive	$\mu \Rightarrow 10,000$ , $\delta \Rightarrow 10,000$ , $\kappa \Rightarrow 10,000$	Neither substituted nor exacerbated withdrawal at 4 and 16 mg/kg
10995	Inactive	32.8	Inactive	Inactive	$\mu = 279$ , $\delta = 2217$ , $\kappa = 564$	Weak inverse dose response
11003	Inactive	Inactive	Inactive	Inactive	$\mu = 867$ , $\delta = 3538$ , $\kappa = 645$	Neither substituted nor exacerbated withdrawal at 4 and 16 mg/kg
11007	Inactive	Inactive	Inactive	Inactive	$\mu = 352$ , $\delta = 1496$ , $\kappa = 97$	Possible slight attenuation of withdrawal at 4 and 16 mg/kg <sup>a</sup>
11011	Inactive	17.6	Inactive	Inactive	$\mu = 568$ , $\delta = 5806$ , $\kappa = 83$	Slight attenuation of withdrawal at 16 mg/kg <sup>b</sup>
11014	22.1 <sup>c</sup>	1.42 <sup>c</sup>	19.1 <sup>c</sup>	-	$\mu = 187$ , $\delta = 2273$ , $\kappa = 283$	Weak, non dose-related attenuation of withdrawal <sup>d</sup>
11021	Inactive	Inactive	Inactive	Inactive	$\mu = 2087$ , $\delta \Rightarrow 10,000$ , $\kappa = 864$	Neither substituted nor exacerbated withdrawal at 4 and 16 mg/kg
11022	Inactive	19.4	Inactive	Inactive	$\mu = 1850$ , $\delta \Rightarrow 10,000$ , $\kappa = 175$	Neither substituted nor exacerbated withdrawal

- a) Behavior at high dose: ataxia, slowing, walking in circles, staggering, spinning while sitting.  
 b) Other effects at 16 mg/kg: ataxia and jaw sag.  
 c) Eyelid ptosis and immobility at 30 mg/kg.  
 d) At high doses, jaw sag, salivation, eyelid ptosis were noted.

TABLE 7. MISCELLANEOUS COMPOUNDS



NIH 10947



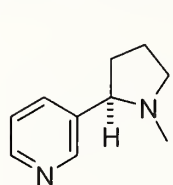
NIH 11016

ANTINOCICEPTIVE/ANTAGONIST ASSAYS IN VITRO MONKEY  
(MOUSE ED<sub>50</sub>/AD<sub>50</sub>, s.c., mg/kg)

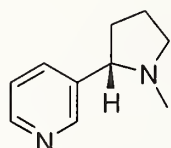
NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10947 <sup>b</sup>	-	iv: 31 <sup>a</sup>	Inactive <sup>a</sup>	-	-	Inverse dose response <sup>a</sup>
11016	Inactive	Inactive	Inactive	Inactive	μ=>10,000 δ=>10,000 κ=>10,000	Feeble attenuation of withdrawal at 16 mg/kg; no attenuation at 32 mg/kg. No precipitation of withdrawal at 32 mg/kg

- a) Previously reported (Coop and Jacobson, 2001). Attenuation of withdrawal at low dose, exacerbation of withdrawal at higher doses. PPQ: Co-administration with ED<sub>25</sub> morphine led to dose-related synergism. Morphine tolerant mice: NIH 10947 + morphine partially restored antinociception.
- b) New Data: In combination with alcohol (GHB in a 12% EtOH solution) (p.o.) mice were ataxic at 30 and 100 mg/kg. Rat Continuous Infusion assays: the animals showed no signs of physical dependence to NIH 10947, nor did NIH 10947 substitute for morphine in morphine dependent rats.

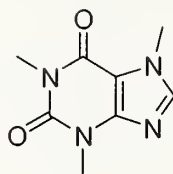
TABLE 8. MISCELLANEOUS (CONTINUED)



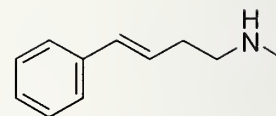
NIH 11017



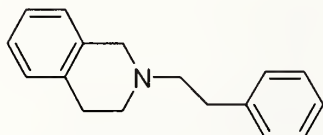
NIH 11018



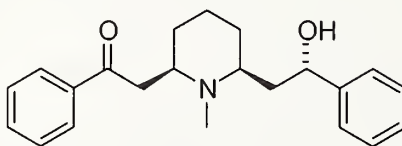
NIH 11019



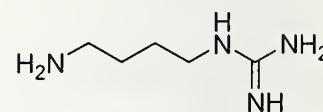
NIH 11024



NIH 11025



NIH 11034



NIH 11035

ANTINOCICEPTIVE/ANTAGONIST ASSAYS  
(MOUSE ED<sub>50</sub>/AD<sub>50</sub>, s.c., mg/kg)

IN VITRO

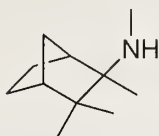
MONKEY

NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
11017 <sup>a</sup>	Inactive	10.23 <sup>b</sup>	Inactive	Inactive	$\mu \Rightarrow 10,000$ $\delta \Rightarrow 10,000$ $\kappa \Rightarrow 10,000$	Reduced withdrawal signs at 1.5 mg/kg, intensified at 6 mg/kg
11018 <sup>c</sup>	16.92 <sup>d</sup>	1.42 <sup>d</sup>	8.91 <sup>d</sup>	Inactive <sup>d</sup>	$\mu \Rightarrow 10,000$ $\delta \Rightarrow 10,000$ $\kappa \Rightarrow 10,000$	Exacerbated withdrawal signs at 0.75 and 3 mg/kg
11019 <sup>e</sup>	Inactive	Inactive	Inactive	Inactive	-	Neither substituted or exacerbated withdrawal
11024 <sup>f</sup>	Inactive	Inactive	Inactive <sup>g</sup>	Inactive	-	Neither substituted or exacerbated withdrawal
11025	Inactive	10.5	Inactive	Inactive	$\mu \Rightarrow 10,000$ $\delta \Rightarrow 10,000$ $\kappa \Rightarrow 10,000$	Displayed delayed attenuation of withdrawal signs at 4 and 16 mg/kg (after 60 mins)
11034	Inactive <sup>h</sup>	3.01	Inactive <sup>h</sup>	Inactive	-	No attenuation of withdrawal at 1 and 4 mg/kg <sup>i</sup>
11035	Inactive	Inactive <sup>j</sup>	Inactive	Inactive	-	A suggestion of attenuation of withdrawal at 6 mg/kg

- a) Previously studied as NIH 9801 (1983): ED<sub>50</sub> HP = 23.2 mg/kg. Inactive in PPQ, TF, and as an antagonist of morphine in TF. Partial suppression of withdrawal signs in withdrawn monkeys.
- b) NTI vs. ED<sub>80</sub> gave erratic antagonism.
- c) Previously studied as NIH 9733 (1984): ED<sub>50</sub> (mg/kg) HP = 2.2; TF = 5.2, PPQ = 1.3. Primary physical dependence in monkey - no withdrawal signs after abrupt withdrawal.
- d) Convulsions were seen in all mouse assays, and were reduced by administration of NTI in TF. NTI had no effect on antinociception in TF
- e) Previously studied as NIH 10613 (1990): Inactive in PPQ and TF.
- f) Previously studied as NIH 10936 (1999): Affinity >10,000 nM for  $\mu$ ,  $\kappa$ , and  $\delta$  receptors.
- g) TF (i.v.) Inactive at 30 mg/kg, but 6/6 mice had clonic convulsions, 3/6 died.
- h) HP: At 30 mg/kg all mice convulsed, and 4/8 died. TF: At 10 mg/kg 5/6 mice convulsed and died
- i) Muscle relaxation and retching was observed
- j) 40% inhibition at 10 mg/kg (s.c.); 50% at 3, 41% at 10, and 53% at 30  $\mu$ g/brain (i.c.v.).



**TABLE 9. EVALUATION OF STIMULANT/DEPRESSANT DRUGS**



CPDD 0059

CPDD#	Discriminative Stimulus Effects in Monkeys. Comparison to Flumazenil & Midazolam (s.c.)	Monkey Self-Administration (iv)	MONKEY DRUG DISCRIMINATION (I.G.)
0059 <sup>a</sup>	-	No reinforcing effects in methohexital trained monkeys	-

- a) Previously reported as NIH 11010 (Coop and Jacobson, 2001): Inactive in TF. PPQ ED<sub>50</sub> = 4.2 mg/kg; neither mecamylamine nor naloxone antagonized NIH 11010 ED<sub>80</sub> in PPQ; 6/6 mice died at 30 mg/kg.

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#### ACKNOWLEDGEMENT

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## EVALUATION OF NEW COMPOUNDS FOR OPIOID ACTIVITY (2001)

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This report contains information on opioid abuse liability evaluations on compounds that have been submitted to the Drug Evaluation Committee of the College and released for publication by the submitters. The information obtained usually involves *in vitro* evaluation in opioid binding assays. In addition, the compounds may be evaluated for discriminative and reinforcing effects. Analgesic and respiratory function assays are also possible. These behavioral assessments are conducted in rhesus monkeys (see Appendix). Usually when limited information is provided (*e.g.*, *in vitro* assessment only), it is because the sample provided by the submitter was insufficient to carry out further evaluation.

The evaluation of new compounds by the programs at the University of Michigan and the Medical College of Virginia was coordinated by Dr. Arthur E. Jacobson, Laboratory of Medicinal Chemistry, NIDDK, National Institutes of Health, Bethesda, MD, and is currently coordinated by Dr. A. Coop, University of Maryland. The compounds, which come originally from pharmaceutical companies, universities, government laboratories, and international organizations are now submitted to Dr. Coop.

At the UM and MCV laboratories, drug samples arrive from the Biological Coordinator with only the following information: (1) an identifying NIH number, (2) molecular weight, (3) solubility information. After the evaluation is complete and the report submitted to Dr. Coop, the submitter is requested to release the chemical structure to include with the evaluation data in the ANNUAL REPORT. The submitter has up to three years before release of the structure is required. When the structure is released all of the data on the compound are reported herein.

### OPIOID RECEPTOR BINDING AND IN VITRO EFFICACY ASSESSMENT

Details of the binding assay been described previously (Lee *et al.*, 1999). Briefly, aliquots of a membrane preparation are incubated with [<sup>3</sup>H]diprenorphine (0.3 nM) in the presence of different concentrations of the drug under investigation at 25° C for 1 hr. Specific, *i.e.*, opioid-receptor-related binding is determined as the difference in binding obtained in the absence and presence of 10 μM naloxone. The potency of the drugs in displacing the specific binding of <sup>3</sup>H-ligand is determined from data using Graphpad Prism (GraphPAD, San Diego, CA) and converted to K<sub>i</sub> values by the method of Cheng and Prussoff (1973). Opioid binding is performed in membranes from C<sub>6</sub> rat glioma cells expressing recombinant μ (rat; Emmerson *et al.*, 1994) or δ (rat; Clark *et al.*, 1997) and CHO cells expressing the recombinant κ (human, Zhu *et al.*, 1997). The affinity (K<sub>d</sub>) values of [<sup>3</sup>H]diprenorphine at the receptors are: μ (0.15 nM); δ (0.45 nM); κ (0.25 nM).

This year, our assays all use recombinant receptors rather than homogenates of monkey brain cortex. The use of recombinant receptors means no cross-reaction with other receptors and allows for direct comparison with cellular functional assays. In the ANNUAL REPORT, the results of the selective binding assays are given as means ± SEM from three separate experiments, each performed in duplicate. K<sub>i</sub> values for standard compounds using recombinant receptors and [<sup>3</sup>H]diprenorphine as radioligand are: μ (DAMGO, 7.6 nM; morphine, 11.2 nM), δ (SNC80, 0.8 nM) and κ (U69593, 0.3 nM). If less than 50% displacement of [<sup>3</sup>H]diprenorphine is seen at 10 μM it is reported as > 10 μM and the percent displacement given in parentheses.

[<sup>35</sup>S]GTPγS assays are carried out using membranes from C6 cells expressing either μ (Emmerson *et al.*, 1996) or δ (Clark *et al.*, 1997) receptors or CHO cells expressing κ receptors (Zhu *et al.*, 1997). Assays are performed as described by Traynor and Nahorski (1995). Values are given as EC<sub>50</sub> with % effect compared to standard agonist (DAMGO, SNC80, or U69593) or as maximal stimulation achieved at 10 μM.

EC<sub>50</sub> values (nM) for standard compounds are as follows:

Mu receptor: morphine (65), DAMGO (34), fentanyl (13)  
 Delta receptor: SNC80 (9), DPDPE (8.3)  
 Kappa receptor: U69593 (31.0),bremazocine (0.5)

DPDPE (60%) and bremazocine (86%) are partial agonists compared with the standards SNC80 and U69593. Morphine and DAMGO give equivalent responses.

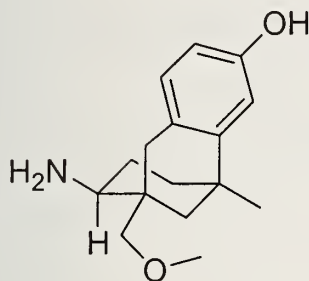
Antagonist activity is given as AD<sub>50</sub> values or as pK<sub>B</sub> values. AD<sub>50</sub> refers to the concentration of test compound that reduces [<sup>35</sup>S]GTPγS binding stimulated by an ED<sub>80</sub> concentration of appropriate agonist (DAMGO, μ; DPDPE, δ; U69593, κ) by 50%. pK<sub>B</sub> is the concentration of antagonist required to shift the dose-effect curve for appropriate agonist by 2-fold. It is a measure of the affinity of the antagonist for a receptor.

### SUMMARY OF TESTS PERFORMED

The compounds that were evaluated at the University of Michigan during the past year are shown in the following Table. Also shown are dates of Reports to the Biological Coordinator, Dr. A.E. Jacobson or Dr. Coop, in which results are reported.

NIH #	Date Submitted to Biological Coordinator	NIH #	Date Submitted to Biological Coordinator
10995	20 March 1998	11014	28 April 2000
10978	22 September 1999	11012	28 April 2000
10978	22 September 1999	11013	28 April 2000
10985	18 April 2000	11014	28 April 2000
10986	18 April 2000	11015	18 January 2001
10987	18 April 2000	11016	18 January 2001
10986	26 April 2001	11017	18 January 2001
10989	18 April 2000	11018	18 January 2001
10990	18 April 2000	11020	14 March 2001
10992	28 February 2000	11020	14 March 2001
10990	28 February 2000	11022	14 March 2001
10995	28 February 2000	11023	14 March 2001
11003	14 April 2000	11025	14 March 2001
11003	14 April 2000	11026	14 March 2001
11005	14 April 2000	11028	14 March 2001
11006	14 April 2000	11037	14 March 2001
11007	14 April 2000		

NIH 10945 (±)-(5*S*,8*S*,9*R*)-8-Amino-3-hydroxy-5,9-methano-9-(methoxymethyl)-5-methylbenzocycoctene



**OPIOID RECEPTOR BINDING (nM)**

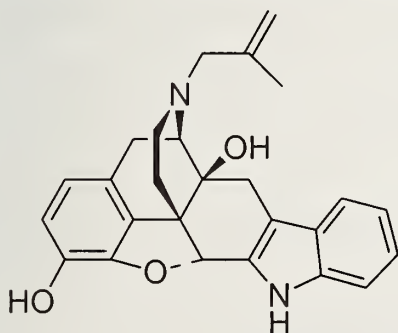
μ-receptor:  $3.7 \pm 0.2$   
δ-receptor:  $156.0 \pm 25$   
κ-receptor:  $6.3 \pm 0.4$

**SUMMARY**

NIH 10945 has a high affinity for both μ and κ opioid receptors. Affinity at δ receptors is over 20-fold less.

\* \* \*

NIH 10978 N-(3-Methylallyl)noroxymorphindole



**OPIOID RECEPTOR BINDING (nM)**

μ receptor:  $430 \pm 60$   
δ receptor:  $0.68 \pm 0.12$   
κ receptor:  $355 \pm 61$

**Agonist Activity**

μ-receptor: <5% stimulation at 10 μM  
δ-receptor: no stimulation at 10 μM  
κ-receptor: no stimulation at 10 μM

**Antagonist Activity**

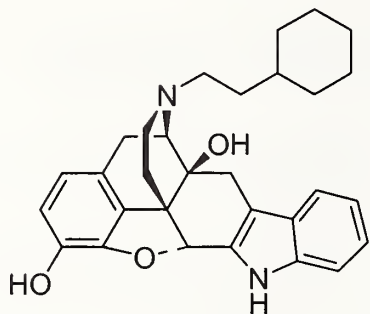
δ-receptor:  $pK_B^* = 8.93$  (8.95 and 8.91)

**SUMMARY**

NIH 10978 has no efficacy at opioid receptors. In binding studies, this compound was highly selective for the δ receptor. It is a high affinity, selective δ antagonist.

\* Determined with a single concentration (10 nM) of antagonist.

NIH 10979 N-Cyclohexylethylmorphine.HCl



OPIOID RECEPTOR BINDING (nM)

$\mu$ receptor:	$7.3 \pm 1.2$
$\delta$ receptor:	$181 \pm 35$
$\kappa$ receptor:	$378 \pm 72$

[<sup>35</sup>S]GTP $\gamma$ S ASSAY (nM)

Agonist Activity

$\mu$ -receptor:  $EC_{50} = 105$  (98.4 and 112) nM: maximal stimulation = 52 (46.2 and 57.5)% \*

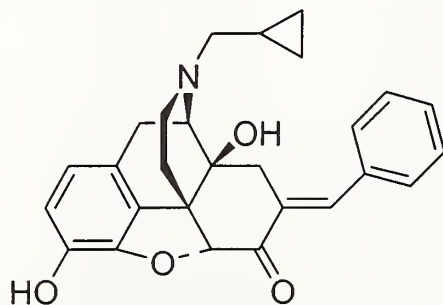
SUMMARY

NIH 10979 has good affinity for  $\mu$  receptors and some selectivity for  $\mu$  over  $\delta$  (25-fold) and  $\kappa$  (52-fold) receptors. It acted as a partial agonist at  $\mu$  receptors

\*Relative to fentanyl = 100%. In this assay, fentanyl has an  $EC_{50}$  of 13 nM.

\* \* \*

NIH 10985 7-Benzylidene-7-dehydronaltrexone.HCl (BNTX; see also NIH 10923)



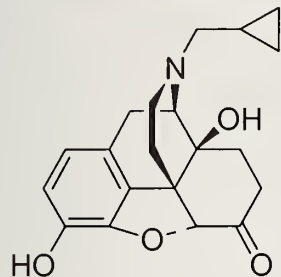
[<sup>35</sup>S]GTP $\gamma$ S ASSAY (nM)

$\mu$  receptor:  $AD_{50} = 4.1 \pm 1.1$

SUMMARY

NIH 10985 is a potent  $\mu$  receptor antagonist.

NIH 10986 Naltrexone.HCl (see also NIH 9930 and NIH 8503)



[<sup>35</sup>S]GTPγS ASSAY (nM)

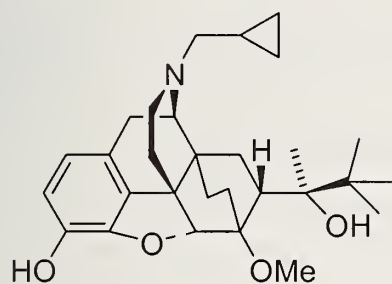
μ receptor: AD<sub>50</sub> = 1.4 ± 0.3

#### SUMMARY

NIH 10986 is a potent μ receptor antagonist.

\* \* \*

NIH 10987 Buprenorphine.HCl (see also NIH 10276 and NIH 8805)



[<sup>35</sup>S]GTPγS ASSAY (nM)

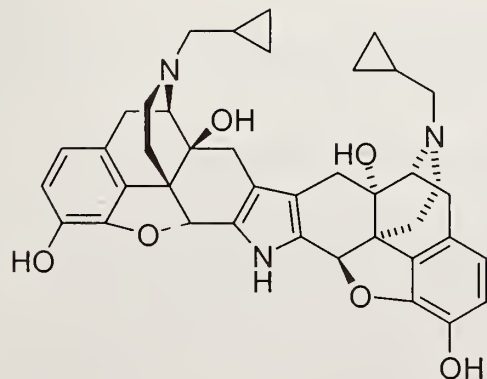
μ receptor: AD<sub>50</sub> = 1.3 ± 0.3  
(maximum inhibition = 67.1 ± 4.0%)

#### SUMMARY

NIH 10987 is a potent μ receptor antagonist. It appears to have some agonist properties.

\* \* \*

NIH 10988 Nor-binaltorphimine.HCl (nor-BNI; see also NIH 10588)



OPIOID RECEPTOR BINDING (nM)

μ-receptor: 0.65 ± 0.20

δ-receptor: 4.0 ± 0.4

κ-receptor: 0.78 ± 0.04

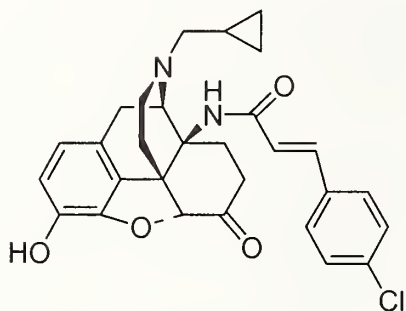
[<sup>35</sup>S]GTPγS ASSAY (nM)

μ-receptor: AD<sub>50</sub> = 11.4 ± 2.5 nM

#### SUMMARY

NIH 10988 had very high affinity for κ and μ opioid receptors and is non-selective between these receptors. It has approximately 5-fold less (but still high) affinity for the δ receptors. It is an antagonist at the μ receptor.

NIH 10989 14β-(p-Chlorocinnamoylamino)-7,8-dihydro-N-cyclopropylmethylnormorphinone mesylate  
(Clocinnamox; see also NIH 10443)



[<sup>35</sup>S]GTPγS ASSAY (nM)

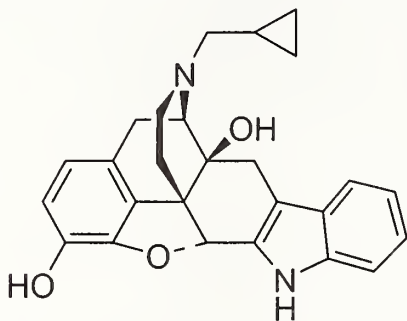
AD<sub>50</sub> = 1.2 ± 0.2 nM

#### SUMMARY

NIH 10989 is a potent μ opioid receptor antagonist.

\* \* \*

NIH 10990 Naltrindole.HCl (see also NIH 10589)



[<sup>35</sup>S]GTPγS ASSAY (nM)

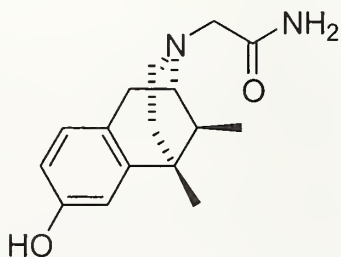
μ receptor: AD<sub>50</sub> = 7.9 ± 2.2

#### SUMMARY

NIH 10990 is a μ opioid receptor antagonist.

\* \* \*

NIH 10992 (+)-(1*S*,5*S*,9*S*)-2-Acetamido-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan



OPIOID RECEPTOR BINDING (μM)

μ-receptor: >10 (0.3% inhibition at 10 μM)

δ-receptor: >10 (16% inhibition at 10 μM)

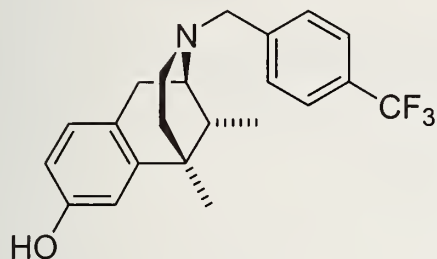
κ-receptor: >10 (2.7% inhibition at 10 μM)

#### SUMMARY

NIH 10992 has no affinity for opioid receptors.



NIH 10994 (-)-(1R,5R,9R)-5,9-Dimethyl-2'-hydroxy-2-(4-trifluoromethylbenzyl)-6,7-benzomorphan oxylate



OPIOID RECEPTOR BINDING (nM)

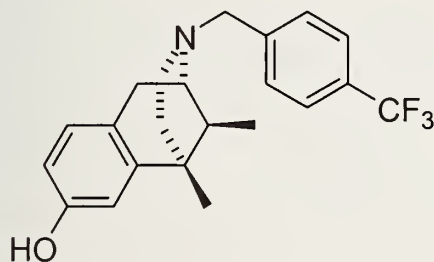
$\mu$ -receptor:  $1435 \pm 333$   
 $\delta$ -receptor:  $>10 \mu\text{M}$  ( $30 \pm 10.4\%$  at  $10\mu\text{M}$ )  
 $\kappa$ -receptor:  $1872 \pm 316$

SUMMARY

NIH 10994 has low affinity for  $\mu$  and  $\kappa$  opioid receptors and very poor affinity at the  $\delta$  receptor.

\* \* \*

NIH 10995 (+)-(1S,5S,9S)-5,9-Dimethyl-2'-hydroxy-2-(4-trifluoromethylbenzyl)-6,7-benzomorphan oxylate



OPIOID RECEPTOR BINDING (nM)

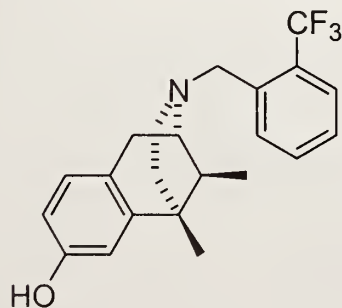
$\mu$ -receptor:  $279 \pm 35.7$   
 $\delta$ -receptor:  $2217 \pm 406$   
 $\kappa$ -receptor:  $564 \pm 93$

SUMMARY

NIH 10995 has low affinity for  $\mu > \kappa \gg \delta$  opioid receptors.

\* \* \*

NIH 11003 (+)-(1S,5S,9S)-5,9-Dimethyl-2'-hydroxy-2-(2-trifluoromethylbenzyl)-6,7-benzomorphan.HCl



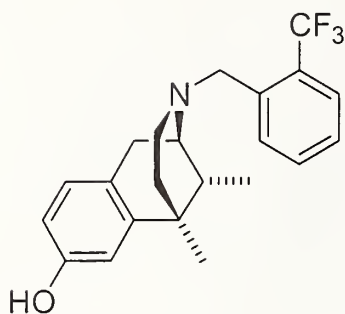
OPIOID RECEPTOR BINDING (nM)

$\mu$ -receptor:  $867 \pm 60$   
 $\delta$ -receptor:  $3538 \pm 936$   
 $\kappa$ -receptor:  $645 \pm 30$

SUMMARY

NIH 11003 has low affinity for opioid receptors in the order  $\kappa = \mu > \delta$ .

NIH 11004 (-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(2-trifluoromethylbenzyl)-6,7-benzomorphan HCl



**OPIOID RECEPTOR BINDING (nM)**

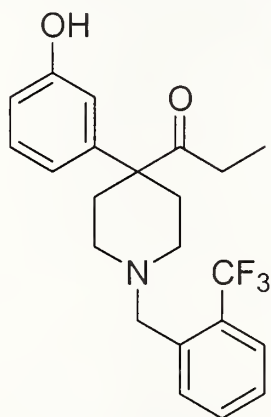
$\mu$ -receptor: 335  $\pm$  95  
 $\delta$ -receptor: 1091  $\pm$  1.8  
 $\kappa$ -receptor: 149  $\pm$  17

**SUMMARY**

NIH 11004 has some affinity for  $\kappa$  receptors >  $\mu$  receptors >  $\delta$  receptors.

\* \* \*

NIH 11005 4-(3-hydroxyphenyl)-4-(1-oxo-propyl)-1-(2-trifluoromethylbenzyl)piperidine.HCl



**OPIOID RECEPTOR BINDING (nM)**

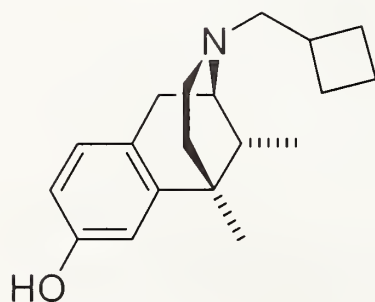
$\mu$ -receptor: 546  $\pm$  101  
 $\delta$ -receptor: 119  $\pm$  18  
 $\kappa$ -receptor: 836  $\pm$  8

**SUMMARY**

NIH 11005 has some affinity for  $\delta$  >  $\mu$  >  $\kappa$  opioid receptors.

\* \* \*

NIH 11006 (-)-(1*R*,5*R*,9*R*)-2-Cyclobutylmethyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.HCl



**OPIOID RECEPTOR BINDING (nM)**

$\mu$ -receptor: 2.9  $\pm$  0.7  
 $\delta$ -receptor: 11.3  $\pm$  3.5  
 $\kappa$ -receptor: 0.74  $\pm$  0.1

NIH 11006 (Continued)

$[^3S]GTP\gamma S$  ASSAY (nM)

$\mu$ -receptor:  $EC_{50} = 2.0 \pm 0.6$  (25.5  $\pm$  10.3% stimulation\*)  
 $\delta$ -receptor:  $EC_{50} = 54.3 \pm 29$  (22.3  $\pm$  6.0% stimulation\*\*)  
 $\kappa$ -receptor:  $EC_{50} = 3.1 \pm 0.9$  (57.9  $\pm$  4.4% stimulation\*\*\*)

SUMMARY

NIH 11006 is a partial agonist at  $\mu$ ,  $\delta$ , and  $\kappa$  receptors. It had similar affinity and potency at  $\mu$  and  $\kappa$  receptors with somewhat less at the  $\delta$  receptor.

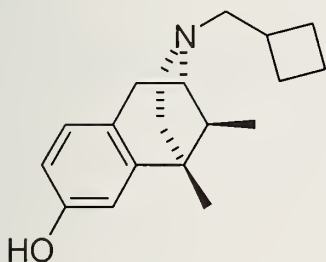
\* relative to fentanyl = 100%. In this assay fentanyl has an  $EC_{50}$  of 13 nM.

\*\* relative to SNC80 = 100%. In this assay, SNC80 has an  $EC_{50}$  of 9 nM

\*\*\*relative to U69593 = 100%. In this assay, U69593 has an  $EC_{50}$  of 31 nM.

\* \* \*

NIH 11007 (+)-(1*S*,5*S*,9*S*)-2-Cyclobutylmethyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.HCl



OPIOID RECEPTOR BINDING (nM)

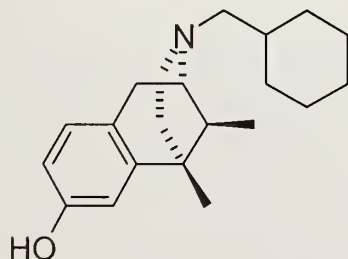
$\mu$ -receptor:  $352 \pm 44$   
 $\delta$ -receptor:  $1496 \pm 161$   
 $\kappa$ -receptor:  $97 \pm 14$

SUMMARY

NIH 11007 has some affinity for the  $\kappa$  opioid receptor, with 4-fold lower affinity for the  $\mu$  receptor and 15-fold lower affinity for the  $\delta$  receptor.

\* \* \*

NIH 11011 (+)-(1*S*,5*S*,9*S*)-2-Cyclohexylmethyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.HCl



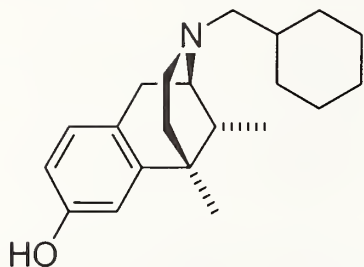
OPIOID RECEPTOR BINDING (nM)

$\mu$ -receptor:  $586 \pm 60$   
 $\delta$ -receptor:  $5806 \pm 750$   
 $\kappa$ -receptor:  $83 \pm 5.8$

SUMMARY

NIH 11011 has some affinity for the  $\kappa$  receptor with 7-fold lower affinity for the  $\mu$  receptor and 70-fold lower affinity for the  $\delta$  receptor.

NIH 11012 (-)-(1*R*,5*R*,9*R*)-2-Cyclohexylmethyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.HCl



**OPIOID RECEPTOR BINDING (nM)**

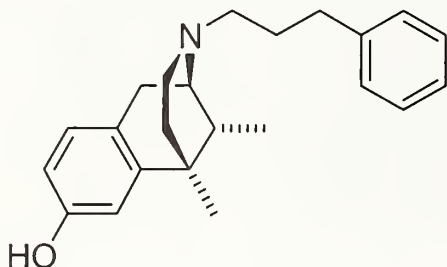
$\mu$ -receptor:	26 $\pm$ 1.8
$\delta$ -receptor:	315 $\pm$ 42
$\kappa$ -receptor:	13 $\pm$ 0.9

**SUMMARY**

NIH 11012 has good affinity for  $\mu$  and  $\kappa$  receptors with approximately 16-fold lower affinity at the  $\delta$  receptor.

\* \* \*

NIH 11013 (-)-(1*R*,5*R*,9*R*)-2-(3-Phenylpropyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.HCl



**OPIOID RECEPTOR BINDING (nM)**

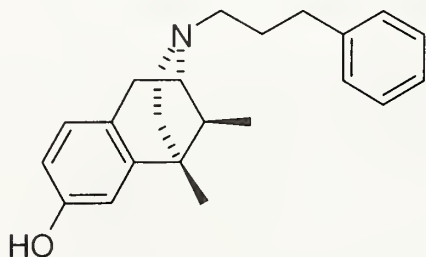
$\mu$ -receptor:	11 $\pm$ 1.5
$\delta$ -receptor:	47 $\pm$ 3.1
$\kappa$ -receptor:	34 $\pm$ 1.8

**SUMMARY**

NIH 11013 has good affinity at all three opioid receptors with very limited selectivity (3-fold) for the  $\mu$  receptor.

\* \* \*

NIH 11014 (+)-(1*S*,5*S*,9*S*)-2-(3-Phenylpropyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.HCl



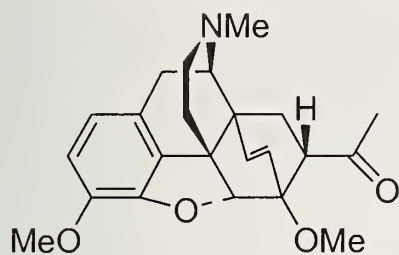
**OPIOID RECEPTOR BINDING (nM)**

$\mu$ -receptor:	187 $\pm$ 18
$\delta$ -receptor:	2273 $\pm$ 213
$\kappa$ -receptor:	283 $\pm$ 26

**SUMMARY**

NIH 11014 has some affinity for  $\mu$  and  $\kappa$  receptors and much lower affinity for the  $\delta$  receptor.

NIH 11015 Thevinone.oxalate (see also NIH 10631)



OPIOID RECEPTOR BINDING (nM)

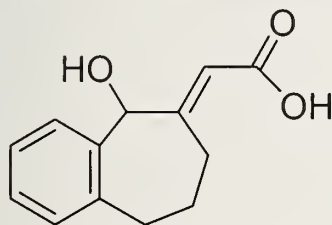
$\mu$ -receptor: 211  $\pm$  19  
 $\delta$ -receptor: 2102  $\pm$  293  
 $\kappa$ -receptor: 6311  $\pm$  925

SUMMARY

NIH 11015 has some affinity for  $\mu$  receptors and much lower affinity for  $\delta$  and  $\kappa$  receptors, giving 10-fold  $\mu/\delta$  selectivity and 30-fold  $\mu/\kappa$  selectivity.

\* \* \*

NIH 11016 NCS-382, sodium salt



OPIOID RECEPTOR BINDING ( $\mu$ M)

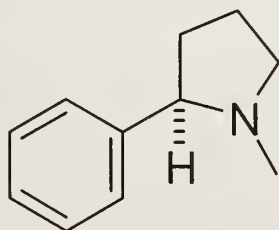
$\mu$ -receptor: >10 (1% inhib. at 10  $\mu$ M)  
 $\delta$ -receptor: >10 (7% inhib. at 10  $\mu$ M)  
 $\kappa$ -receptor: >10 (3% inhib. at 10  $\mu$ M)

SUMMARY

NIH 11016 has no affinity for opioid receptors.

\* \* \*

NIH 11017 (*R*)-(+)-Nicotine di-*d*-tartrate (see also NIH 9801)



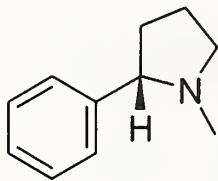
OPIOID RECEPTOR BINDING ( $\mu$ M)

$\mu$ -receptor: >10 (21% inhib. at 10  $\mu$ M)  
 $\delta$ -receptor: >10 (15% inhib. at 10  $\mu$ M)  
 $\kappa$ -receptor: >10 (10% inhib. at 10  $\mu$ M)

SUMMARY

NIH 11017 has no appreciable affinity at opioid receptors.

NIH 11018 (S)-(-)-Nicotine di-l-tartrate (see also NIH 9733)



**OPIOID RECEPTOR BINDING ( $\mu\text{M}$ )**

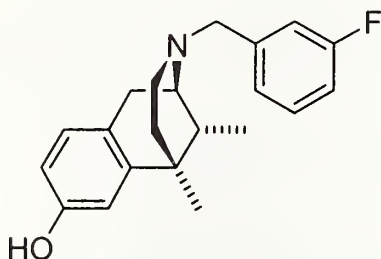
$\mu$ -receptor:  $>10$  (20% inhib. at  $10 \mu\text{M}$ )  
 $\delta$ -receptor:  $>10$  (10% inhib. at  $10 \mu\text{M}$ )  
 $\kappa$ -receptor:  $>10$  (14% inhib. at  $10 \mu\text{M}$ )

**SUMMARY**

NIH 11018 has no appreciable affinity for opioid receptors.

\* \* \*

NIH 11020 (-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2-(3-fluorobenzyl)-2'-hydroxy-6,7-benzomorphan.oxylate



**OPIOID RECEPTOR BINDING (nM)**

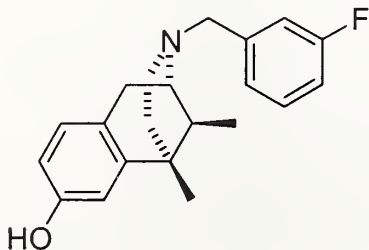
$\mu$ -receptor:  $16.3 \pm 1.4$   
 $\delta$ -receptor:  $351 \pm 29$   
 $\kappa$ -receptor:  $7.9 \pm 0.2$

**SUMMARY**

NIH 11020 has good affinity for  $\kappa$  and  $\mu$  receptors and much less affinity at the  $\delta$  receptor. It had only a little  $\kappa/\mu$  selectivity.

\* \* \*

NIH 11021 (+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2-(fluorobenzyl)-2'-hydroxy-6,7-benzomorphan.oxalate



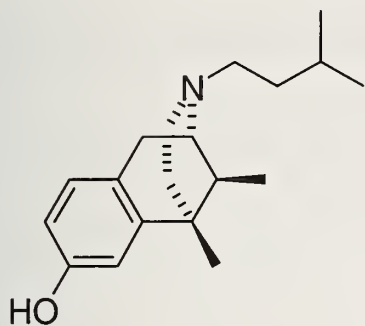
**OPIOID RECEPTOR BINDING (nM)**

$\mu$ -receptor:  $2087 \pm 868$   
 $\delta$ -receptor:  $>10 \mu\text{M}$  (33% inhib. at  $10 \mu\text{M}$ )  
 $\kappa$ -receptor:  $864 \pm 236$

**SUMMARY**

NIH 11021 has no appreciable affinity for the  $\delta$  opioid receptor and very low affinity for  $\kappa > \mu$ .

NIH 11022 (+)-(1*S*,5*S*,9*S*)-2-(3-Methylbutyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.oxalate



**OPIOID RECEPTOR BINDING (nM)**

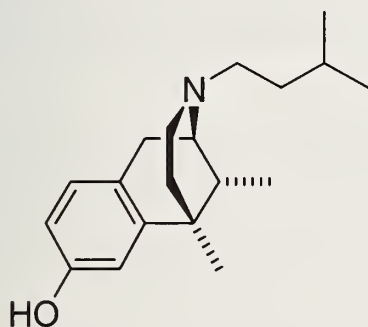
$\mu$ -receptor: 1850  $\pm$  729  
 $\delta$ -receptor: >10  $\mu$ M (43% inhib. at 10  $\mu$ M)  
 $\kappa$ -receptor: 175  $\pm$  51.9

**SUMMARY**

NIH 11022 has low affinity for  $\kappa > \mu$  opioid receptors. It has very low affinity for  $\delta$ -opioid receptors.

\* \* \*

NIH 11023 (-)-(1*R*,5*R*,9*R*)-2-(3-Methylbutyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.oxalate



**OPIOID RECEPTOR BINDING (nM)**

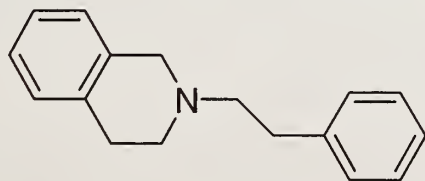
$\mu$ -receptor: 16.4  $\pm$  3.1  
 $\delta$ -receptor: 36.7  $\pm$  0.5  
 $\kappa$ -receptor: 7.3  $\pm$  1.9

**SUMMARY**

NIH 11023 has good affinity for all three opioid receptors with little selectivity (5-fold preference for  $\kappa$  over  $\delta$  and 2-fold preference for  $\kappa$  over  $\mu$ ).

\* \* \*

NIH 11025 2-(2-phenethyl)-1,2,3,4-tetrahydroisoquinoline.oxalate



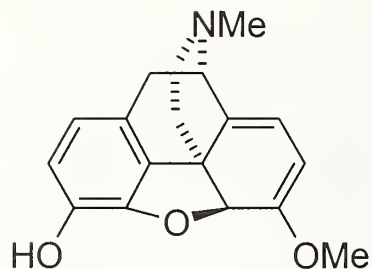
**OPIOID RECEPTOR BINDING ( $\mu$ M)**

$\mu$  receptor: >10 (24% inhib. at 10  $\mu$ M)  
 $\delta$  receptor: >10 (21% inhib. at 10  $\mu$ M)  
 $\kappa$  receptor: >10 (27.5% inhib. at 10  $\mu$ M)

**SUMMARY**

NIH 11025 has no appreciable affinity for any of the opioid receptor types.

NIH 11026 (+)-Oripavine.oxalate



**OPIOID RECEPTOR BINDING (nM)**

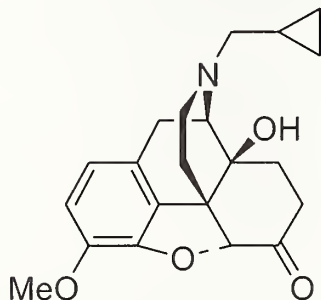
$\mu$  receptor:  $806 \pm 85$   
 $\delta$  receptor:  $>10 \mu\text{M}$  (36.8% inhib. at  $10 \mu\text{M}$ )  
 $\kappa$  receptor:  $>10 \mu\text{M}$  (40.3% inhib. at  $10 \mu\text{M}$ )

**SUMMARY**

NIH 11026 has no appreciable affinity for  $\delta$  or  $\kappa$  opioid receptors, and low affinity for  $\mu$  receptors.

\* \* \*

NIH 11028 3-O-Methylnaltrexone.HCl



**OPIOID RECEPTOR BINDING (nM)**

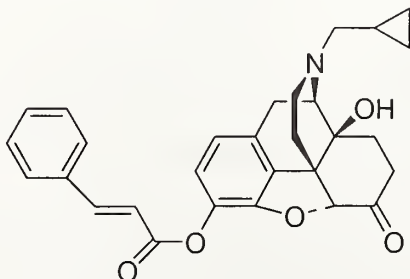
$\mu$  receptor:  $30.8 \pm 6.2$   
 $\delta$  receptor:  $589 \pm 58$   
 $\kappa$  receptor:  $95.2 \pm 12.7$

**SUMMARY**

NIH 11028 has some affinity for  $\mu$  and  $\kappa$  opioid receptors and less affinity for  $\delta$  receptors.

\* \* \*

NIH 11037 3-O-Cinnamoylnaltrexone.HCl



**OPIOID RECEPTOR BINDING (nM)**

$\mu$  receptor:  $18.4 \pm 8.1$   
 $\delta$  receptor:  $385 \pm 87$   
 $\kappa$  receptor:  $30.7 \pm 6.2$

**SUMMARY**

NIH 11037 has affinity for  $\mu$  and  $\kappa$  receptors, but  $> 10$ -fold lower affinity for  $\delta$  receptors.



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## APPENDIX

The University of Michigan laboratories also offer the following tests:

### DRUG DISCRIMINATION IN RHESUS MONKEYS

We currently use three groups of monkeys to test the discriminative stimulus effects of submitted drugs: one of these groups discriminates the administration of the  $\kappa$  agonist ethylketazocine (EKC); a second group discriminates the  $\mu$  agonist alfentanil or fentanyl; a third group is treated daily with morphine and discriminates the opioid antagonist naltrexone.

The procedures used with the EKC-trained monkeys have been described by Bertalmio *et al.* (1982). The monkeys are removed from their home cages each day and seated in primate restraining chairs. These chairs are placed in chambers equipped with two response levers, several stimulus lights and a cup to receive Noyes, banana-flavored pellets. These monkeys are required to make 100 consecutive responses on the correct one of the two levers and receive ten 300-mg food pellets. The right lever is correct if they were given a subcutaneous injection of 0.0032 mg/kg EKC immediately prior to the start of the cycle. The left lever is designated correct if they were given a sham injection before the start of the cycle. Each cycle lasts 15-min and consists of an initial 10-min black out period followed by a period of as long as 5 min, during which a blue light is illuminated in the chamber and the monkey can respond for food. If the food pellets are delivered before the 5 min period is completed, the lights are extinguished for the remainder of this time. Typically, a daily session consists of several 15 min cycles. During a training session, if EKC is given, it is given on the penultimate cycle of that session. Responding on the drug-appropriate lever is reinforced during that cycle and on the subsequent, final cycle of the day. These last two cycles may be preceded by from zero to four sham cycles on a training day. A training session of six sham cycles is also scheduled from time to time.

With this type of multiple, discrete-cycle training, the animals can be tested with a cumulative dosing procedure. On a test session, the first cycle is preceded by an injection of saline, and prior to subsequent cycles, increasing, cumulative doses of the test drug are administered. One hundred consecutive responses on either lever are reinforced throughout the test session. The test drug is administered in increasing doses until the monkey either responds on the drug-appropriate lever, the response rate falls to less than half of the saline-control rate, or six cycles are given. In the latter situation, it is assumed that the selected dose range is too low, and the test is continued at higher doses on the next test session. Each test session is preceded and followed by a training session. The criterion for satisfactory performance must be met on each training session that is followed by a test session. This criterion is that at least 90% of the responses during each cycle of a training session must be on the injection-appropriate lever, either sham or EKC.

The procedure for the alfentanil-trained monkeys is similar, but not identical. These animals are also trained and tested in a discrete, multiple-cycle procedure. The main difference between the alfentanil procedure and the EKC procedure is that the alfentanil monkeys are required to make 20 rather than 100 responses, and they receive a single pellet for correct responses. They can receive as many as 10 pellets during the 5-min, food-availability period of each cycle, but each pellet is delivered after 20 responses. Because in this procedure, monkeys can switch from one lever to another following the delivery of food, an additional criterion is added for satisfactory performance. In addition to making 90% or more of their responses on the correct lever, the monkeys must make fewer than 20 responses on the incorrect lever prior to delivery of the first food pellet of each cycle. Tests of the discriminative stimulus effects of submitted drugs in the alfentanil-trained monkeys are also done using a cumulative dosing procedure with dosing criteria identical to those used in the EKC-trained monkeys.

The procedure for studying discriminative stimulus effects in morphine-treated monkeys has been described previously (France and Woods, 1989). Daily sessions are comprised of a 10-min time out during which lever presses have no programmed consequence and a 5-min response period during which green stimulus lights are illuminated and signal the activation of a schedule of stimulus-shock termination. Sessions consist of between two and six discrete, 15-min cycles with each cycle. Under these experimental conditions electric shock is scheduled to be delivered to the subject's feet every 15 seconds; monkeys can terminate the lights and postpone scheduled shocks for 30 seconds by pressing five times consecutively (*i.e.*, fixed-ratio 5) the lever appropriate for the solution

administered during the first minute of the time out (left lever, saline; right lever, naltrexone). Monkeys receive an injection of saline (0.1 ml/kg) or drug (0.01 mg/kg naltrexone) during the first minute of each time out. On drug training days a single injection of naltrexone is administered during one time out and for that cycle and all subsequent cycles on that day only responding on the right lever postpones shocks. A variable number of saline cycles (0-5) precede the naltrexone cycle and on some days saline is administered during the time out of all cycles. Under these conditions monkeys switch their response choice from the saline lever to the naltrexone lever with complete generalization occurring in all three subjects at a dose of 0.01 mg/kg. Responding on the naltrexone lever is accompanied by other behavioral effects indicative of opioid withdrawal (*e.g.*, irritability, miosis, salivation). Moreover, when saline is substituted for the daily injection of 3.2 mg/kg of morphine monkeys respond predominantly on the naltrexone lever and show directly observable signs of withdrawal; the discriminative stimulus and other effects produced by morphine abstinence are reversed by some opioid agonists (*e.g.*, alfentanil; France and Woods, 1989; France *et al.*, 1990).

For test sessions increasing doses of drug are administered during the first minute of consecutive time outs and five consecutive responses on either lever postpone shocks. In monkeys that receive 3.2 mg/kg of morphine 3 hours earlier, increasing doses of a test compound are administered up to doses that produce an average of at least 80% responding on the naltrexone lever or to doses that disrupt responding and result in the delivery of electric shock. Drugs that do not substitute for naltrexone (*i.e.*, precipitate withdrawal) are also studied for their ability to reverse responding on the naltrexone lever in morphine-abstinent (*i.e.*, withdrawn) subjects. Test compounds are studied using a cumulative-dosing procedure in morphine-abstinent monkeys up to doses that reverse completely responding on the naltrexone lever (<20%) or to doses that disrupt responding. Some compounds that substitute for naltrexone also are studied for their capacity to prevent the effects of cumulative doses of opioid agonists. Monkeys that receive saline three hours earlier, rather than the daily injection of morphine, receive saline (control) or a single injection of test compound during the first cycle and increasing doses of agonist (alfentanil or morphine) during subsequent cycles. Agonists are administered up to doses that produce a switch from the naltrexone lever to the saline lever or to doses that disrupt responding and result in the delivery of electric shock.

#### **THERMAL ANALGESIA IN RHESUS MONKEYS**

The tail withdrawal procedure used to study analgesic effects of test compounds in rhesus monkeys has been described previously (Dykstra and Woods, 1986). Monkeys are restrained loosely at the neck and arms while seated in Plexiglas primate chairs. For tests of tail withdrawal latency, the lower 10-12 cm of the shaved tail is immersed in a thermos containing water at 40E, 50E, or 55E C and the latency until the tail is withdrawn from the thermos is recorded for each monkey at each temperature. When the tail is not withdrawn within 20 seconds (cut-off latency) the experimenter removes the thermos and a latency of 20 seconds is recorded. Experimental sessions begin with several exposures to 40EC water. Four or five monkeys are tested consecutively and the time between tail immersions for individual monkeys is 5 minutes. Generally, 40E C water does not produce tail withdrawal in rhesus monkeys (Dykstra and Woods, 1986); however, if a monkey fails to keep its tail in 40E C water for 20 seconds on at least 3 of 4 immersions, that animal is not tested further for that particular session. In a subsequent pre-test component, tails are immersed in 40E, 50E, and 55E C water. The order in which the three temperatures are presented is varied among subjects. If the latencies for tail withdrawal in the pre-test component are at or near 20 seconds for 40E C water and less than 5 seconds for 55E C water, monkeys receive the test compound. The test is identical to the pre-test, except that monkeys receive s.c. injections of drug 10 minutes prior to tail immersion. The time between immersions for individual subjects is 5 minutes or less and the order in which temperatures are presented varies among subjects and across cycles. The interinjection interval typically is 30 minutes and between four and six doses are studied in a single experiment using the cumulative dosing procedure. For some studies a single dose of an opioid antagonist is administered prior to the test compound and for other studies a single dose of test compound is administered prior to increasing doses of a  $\mu$  (*e.g.*, alfentanil) or  $\kappa$  (*e.g.*, U-50,488) opioid agonist.

#### **RESPIRATORY STUDIES IN RHESUS MONKEYS**

The effects of test compounds on ventilatory function are studied in rhesus monkeys breathing air or 5% CO<sub>2</sub> in air (France and Woods, 1990; Howell *et al.*, 1988). Monkeys are restrained at the neck and waist while seated in a Plexiglas primate chair. Normal air or 5% CO<sub>2</sub> in air is delivered at a rate of 10 l/min into a sealed helmet placed over the subject's head. Changes in pressure within the helmet are measured and recorded by a transducer and a

microprocessor, and are transformed according to known standards to frequency of respiration ( $f$ ) in breaths/minute and to tidal volume ( $V_T$ ) in ml/inspiration. Data are recorded continuously during 23-minute exposures to air alternating with 7-minute exposures to  $CO_2$ . The last 3 minutes of exposure to  $CO_2$  are used for data analyses and are compared to the last 3 minutes of exposure to air only. Increasing doses of drug are administered during the first minute of consecutive time outs so that the interinjection interval is 30 minutes. For some studies a single injection of an opioid antagonist is administered prior to increasing doses of a test compound and for other studies a single injection of test compound is administered prior to cumulative doses of a standard compound (*e.g.*, alfentanil).

### **SELF-ADMINISTRATION BY MONKEYS**

Tests of self-administration determine the ability of the drug to maintain responding in monkeys trained to self-inject codeine. Each of at least three monkeys is studied with saline as a negative control and a number of doses of the test compound until a maximum rate of responding was obtained or until, in the absence of evidence of a reinforcing effect, observable changes in behavior are produced by the compound.

The schedule of intravenous drug delivery is a fixed-ratio 30; when a light above a lever is illuminated, the 30th response produce an intravenous drug injection accompanied by another light that is illuminated during drug delivery. After each injection, a 45 sec timeout period occurs. A component of the session ends after 20 injections have been received or 25 min have passed, whichever occurs first. Different doses of the drug are available during each of four components of a session. Other procedural details are given in Winger *et al.* (1989).

## DEPENDENCE STUDIES OF NEW COMPOUNDS IN THE RHESUS MONKEY, RAT AND MOUSE (2001)

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All compounds, except ( $\gamma$ )-hydroxybutyric acid, caffeine, lobeline and agmatine were unknown to us when submitted by the Biological Coordinator, Dr. Andrew Coop of University of Maryland, School of Pharmacy. These studies were conducted under the auspices of the Drug Evaluation Committee in association with the College on Problems of Drug Dependence. See summary of new data in Table 1.

### Dependence-Liability Studies in Rhesus Monkeys

*Substitution-for-Morphine (SDS) Test.* Male and female rhesus monkeys (*M. mulatta*) weighing 2.5-7.5 kg were used, and they received 3 mg/kg, s.c., of morphine-SO<sub>4</sub> every 6 hr. All the animals had received morphine for at least 3 months and were maximally dependent on morphine (Seevers and Deneau 1963). A minimal 2-week recuperation period was allowed between tests. At least 3 monkeys/dose were used. The assay (Aceto and co-workers, 1977 and 1978) was initiated by a subcutaneous injection of the test drug or control substances (morphine and vehicle) into animals in a group that had not received morphine for 14-15 hr and showed definite signs of withdrawal. Each animal was randomly chosen to receive one of the following treatments: a) a dose of the compound under investigation; b) morphine control, 3.0 mg/kg; and c) vehicle control, 1 ml/kg. The animals were scored for suppression of withdrawal signs during a 2.5-hr observation period. The observer was "blind" regarding the choice of treatments. At the end of the study, the data were grouped according to dose and drug. The mean cumulative score  $\pm$  SEM was calculated and the data illustrated in figure form. If indicated, the data were analyzed using the Kruskal-Wallis ANOVA and post hoc Mann-Whitney U-Tests.

*Precipitated-Withdrawal (PPT-W) Test.* This evaluation was done under the same conditions as described above, except that the animals were administered a test compound 2-3 hr after the last dose of morphine. These animals were not in withdrawal. Naloxone HCl (0.05 mg/kg, s.c.) served as the positive control.

*Primary-Physical-Dependence (PPD) Study.* Drug-naive monkeys were medicated with drug, using escalating dose regimens, periodically challenged with naloxone or placed in abrupt withdrawal. They were observed for overt behavioral signs during drug administration and when they were challenged with the antagonist, naloxone, or abruptly withdrawn from the drug.

### Rat-Infusion Studies

The continuous-infusion method was reported by Teiger (1974) and certain modifications are indicated as follows. Rats were anesthetized after which each was fitted with a specially prepared cannula, which was passed subcutaneously from the nape of the neck to the lateral side of the lower abdomen and then inserted into the peritoneal cavity. The cannula was anchored at both ends with silk sutures and attached to a flow-through swivel mechanism, which allowed the animal to move about in the cage and eat and drink normally. The swivel was connected to a syringe, which was attached to a syringe pump. The animals received 7-10 ml of solution every 24 hr.

TABLE 1 SUMMARY OF NEW DATA

NIH No.	Chemical Name or Generic Class	MOUSE					RAT		MONKEY		
		TF	TFvsM	PPQ	HP	pA2	SM	PPD	SDS	PPT-W	PPD
10497	Morphine	T <sup>a</sup>									
10945	Methanobenzocyclooctene	T	T	T <sup>b</sup>	T				T		
10947	4-Hydroxybutyric acid						T	T			
10978	Noroxymorphindole	T	T	T	T				T		
10979	Noroxymorphindole	T <sup>c</sup>	T	T	T						
10992	(+)-6,7,-benzomorphan	T	T	T	T				T		
10994	(-)-6,7,-benzomorphan	T	T	T	T				T		
10995	(+)-6,7,-benzomorphan	T	T	T	T				T		
11003	(+)-6,7,-benzomorphan	T	T	T	T				T		
11004	(-)-6,7,-benzomorphan	T	T	T	T				T		
11005	4-Phenylpiperidine	T	T	T	T				T		
11006	(-)-6,7,-benzomorphan	T <sup>d</sup>	T	T	T				T		
11007	(+)-6,7,-benzomorphan	T	T	T	T				T		
11012	(+)-6,7,-benzomorphan	T	T	T	T <sup>e</sup>				T		
11013	(-)-6,7,-benzomorphan	T	T	T	T				T		
11014	(+)-6,7,-benzomorphan	T	T	T	T				T		
11015	Thevinone	T <sup>f</sup>	T	T	T				T		
11016	GHB antagonist	T	T	T	T				T	T	
11017	(+)-Nicotine	T	T	T <sup>g</sup>	T				T		
11018	9-0-Nicotine	T	T	T <sup>h</sup>	T				T		
11019	Caffeine	T	T	T	T				T		
11020	(-)-6,7,-benzomorphan	T	T	T	T				T		
11021	(+)-6,7,-benzomorphan	T	T	T	T				T		
11022	(+)-6,7,-benzomorphan	T	T	T	T				T		
11023	(-)-6,7,-benzomorphan	T	T	T	T				T		

Table 1 Continued

NIH No.	Chemical Name or Generic Class	MOUSE					RAT		MONKEY		
		TF	TFvsM	PPQ	HP	pA2	SM	PPD	SDS	PPT-W	PPD
11024	Metanicotine	T	T	T	T				T		
11025	Tetrohydroquinoline	T	T	T	T				T		
11026	(+)-Oripavine	T	T	T	T				T		
11028	3-O-methylnaltrexone	T <sup>a</sup>	T	T	T						
11034	L-Lobeline	T	T	T	T				T		
11035	Agmatine	T	T	T	T				T		
11037	3-O-Cinnamoylnaltrexone	T	T	T	T					T	

T = Test Performed

<sup>a</sup>Comparison of the antinociceptive effects of three samples of NIH 10497 in TF. <sup>b</sup>Special: Naloxone vs ED80 of NIH 10945 in PPQ. <sup>c</sup>Special: Naloxone vs ED80 of NIH 10979 in TF. <sup>d</sup>Special: Naloxone vs ED80 of NIH 11006 in TF. <sup>e</sup>Special: Naltrindole vs ED80 of NIH 11012 in PPQ. <sup>f</sup>Special: Naloxone,  $\beta$ -FNA, nor-BNI and naltrindole vs ED80 of NIH 11015 in TF. <sup>g</sup>Special: Naltrindole vs ED80 of NIH 11017 in PPQ. <sup>h</sup>Special: Naltrindole vs ED80 of NIH 11018 in TF. <sup>i</sup>Special: NIH 11028 vs ED80 of Morphine (s.c. and p.o.) in TF.

*Substitution-for-Morphine (SM) Test.* The rats received morphine·SO<sub>4</sub> (50 mg/kg/24 hr on the first day, 100 mg/kg/24 hr on the second day, and 200 mg/kg/24 hr from days 3 and 4). Then, a test drug was substituted for 2 days. The morphine controls received an infusion of sterile water for injection. The animals were observed for changes in body weight and for behavioral-withdrawal signs for 0.5 hr at 6, 24, 48, 72 and/or 96 hr after stopping the infusion of morphine.

*Primary-Physical-Dependence (PPD) Study.* The rats received test compound, as specified above, for 4-6 days and then, were placed in abrupt withdrawal and observed for overt behavioral signs.

### **Mouse-Antinociception Tests**

Male mice, weighing 20-30 g, were used. All drugs were dissolved in distilled water or in the vehicle indicated and injected subcutaneously (s.c.). At least three doses were tested, and 6-10 animals per dose were used. When applicable, ED<sub>50</sub>'s were calculated by using computerized probit analysis. The results obtained with reference compounds are summarized in Table 2. Occasionally, when requested, drugs were given orally (p.o.) or intravenously (i.v.) and the pretreatment times are indicated in the text.

*Tail-Flick (TF) and (TF vs M) Assays.* The procedure and modifications were described (D'Amour and Smith 1941 and Dewey *et al.* 1970 and 1971) in the literature. Briefly, the mouse's tail was placed in a groove, which contained a slit under which was located a photoelectric cell. When the heat source of noxious stimulus was turned on, the heat focused on the tail, and the animal responded by flicking its tail out of the groove. Thus, light passed through the slit and activated the photocell, which, in turn, stopped the recording timer. The heat source was adjusted to produce tail flick of 2-4 sec under control conditions. Mice were injected with drug or vehicle and tested 20 min later. In three assays for antagonism of the antinociceptive effect, the potential antagonists were administered 10 min before the agonist, and evaluation occurred 20 min later.

*Phenylquinone Abdominal-Stretching (PPQ) Assay.* The procedure was reported previously (Pearl and Harris, 1966). The mice were injected with test drug and 10 min later received 2.0 mg/kg intraperitoneally (i.p.) of a freshly prepared paraphenylquinone (PPQ) solution. The mice were then placed in cages in groups of two each. Ten min after the PPQ injection, the total number of stretches per group were counted over a 1-min period. A stretch was characterized by an elongation of the mouse's body, development of tension in the abdominal muscles, and extension of the forelimbs. The antinociceptive response was expressed as the percent inhibition of the PPQ-induced stretching response.

*Hot-Plate (HP) Assay.* The method was also reported previously (Eddy and Leimbach, 1953 and Atwell and Jacobson, 1978). The hot plate was held at 55°C. Mice were placed on the hot plate and activity was scored if the animal jumped or licked its paws after a delay of 5 sec or more, but no more than 30 sec beyond the control time.



Table 2

Comparative Data (ED50, mg/kg s.c.) [95% C.L.] of Selected Standards in 4 Mouse Agonist-Antagonist Tests

Drug	Tail-Flick	Tail-Flick Antagonist	Phenylquinone	Hot-Plate
Pentazocine	15% at 10.0	18	1.7 (12-26)	13% at 30.0 (1.0-2.5)
Cyclazocine	17% at 1.0 <sup>a</sup>	0.03 (0.02-0.78)	0.01 (0.005-0.03)	25% at 9.0
Nalorphine-HCl	None at 10.0	2.6 (0.7-1.0)	0.6 (0.03-1.44)	13% at 30.0
Naloxone-HCl	None at 10.0	0.04 (0.0-0.09)	No Activity	----
Naltrexone-HCl	None at 10.0	0.007 (.002-0.02)	No Activity	----
Morphine-SO <sub>4</sub> <sup>b</sup>	1.92 (0.89-4.14)	Inactive	0.4 <sup>b</sup> (0.2-0.8)	0.85 (0.39-1.86)
Codeine-P0 <sub>4</sub>	----Inactive	8.25 (5.12-13.29)	6.4 (2.4-16.8)	
Meperidine-HCl	8.37 (4.59-15.27)	Inactive (1.18-11.7)	----	4.6

<sup>a</sup>Mice were ataxic at 3.0 and 10.0 mg/kg but there was no further increase in reaction time<sup>b</sup>ICR - Harlan-Sprague-Dawley Inc.

*Calculation of Apparent pA<sub>2</sub>.* Using the tail-flick or PPQ assay, the apparent pA<sub>2</sub> and 95% confidence limits were calculated using Schild and constrained plots as described in Tallarida and Murray (Manual of Pharmacologic Calculations with Computer Programs, 2nd ed., Springer Verlag, NY, 1987).

Briefly, mice were pretreated with vehicle or various doses of antagonist followed 10 min later by an injection of agonist. The mice were tested 30 min after receiving the antagonist. Dose-response lines for antinociception were plotted using at least 3 doses of each opioid agonist in the presence of vehicle or one of the selected doses of antagonist. ED50s were estimated according to the method of Litchfield and Wilcoxon (J. Pharmacol. Exp. Ther., 96, 399, 1949). Each dose ratio (x) was calculated by dividing the ED50 of the opioid in the presence of a given dose of antagonist by that of the agonist alone. Log (x-1) was plotted against <sup>a</sup>Negative logarithm of the molar concentrations of antagonist required to produce a two-fold shift of the agonist dose-response curve to the right. Competitive antagonism can be assumed when slope = -1. pA<sub>2</sub> provides a measure of the relative potency and affinity of the antagonist. When the slope differs significantly from unity, this may indicate non-equilibrium conditions, interactions with multireceptors, receptor sensitization, precoupling mechanisms, or multiple drug properties. With a constrained plot, the slope of the regression line is restricted to slope of -1.

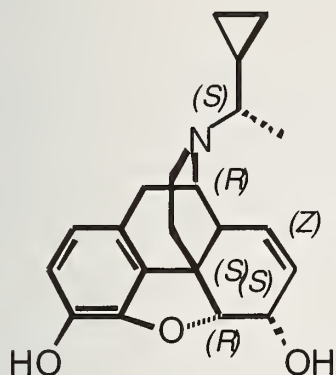
*Special Intracerebroventricular Tail-Flick and PPQ Assays.* In order to develop an in-vivo agonist and antagonist model to correlate with the in-vitro binding data of the various opioid receptor types (mu, kappa and delta), we chose the mouse Tail-Flick and PPQ tests and a variety of routes of administration. The intracerebroventricular (i.c.v.) route was chosen to accommodate the fact that no delta agonist is available which is active by peripheral routes of administration.

Briefly, mice were pretreated with vehicle or various doses of antagonist followed 10 min later by an injection of agonist. The mice were tested 30 min after receiving the antagonist. Dose-response lines for antinociception were plotted using at least 3 doses of each opioid agonist in the presence of vehicle or one of the selected doses of antagonist. ED50s were estimated according to the method of Litchfield and Wilcoxon (J. Pharmacol. Exp. Ther., 96, 399, 1949). Each dose ratio ( $x$ ) was calculated by dividing the ED50 of the opioid in the presence of a given dose of antagonist by that of the agonist alone.  $\log(x-1)$  was plotted against the negative logarithm of the molar dose of the antagonist. At least 3 logs ( $x-1$ ) were plotted. The  $pA_2$  values for the antagonists were calculated from the point of intersection of the regression line with the abscissa. See Table 3 for summary of results.

Table 3. Apparent  $pA_2$  values<sup>a</sup> using the mouse tail-flick assay

<u>Treatment</u> Antagonist/Agonist	<u>Schild Plot</u> $pA_2$ (95% C.L.) Slope	<u>Constrained Plot</u> $pA_2$ (95% C.L.)
1) Naloxone/Morphine	7.2 (7.0-7.4)-1.2	7.3 (7.1 - 7.6)
2) Naloxone/Sufentanil	7.0 (6.5 - 7.5)-1.0	7.0 (6.8 - 7.1)
3) Naloxone/Mirfentanil	7.6 (7.3 - 8.0)-0.7	7.2 (6.9 - 7.5)
4) Naloxone/NIH 10672 (Enadoline) (selective kappa agonist)	6.1 (5.6 - 6.6)-1.2	6.6 (6.3 - 7.0)
5) Naloxone/U-50,488 (kappa agonist)	6.6 (6.3 - 6.9)-1.1	6.2 (5.9 - 7.3)
6) Naloxone/(-)-Nicotine	5.3 (5.3-5.3)-0.5	-
7) Nalmefene/Morphine	8.0 (7.6 - 8.3)-1.1	8.0 (7.7 - 7.6)
8) Naltrexone/Morphine	7.7 (4.9 - 10.5)-0.8	7.6 (7.1 - 8.3)
9) (-)-Quadazocine/Morphine	6.8 (6.7 - 7.0)-0.9	6.8 (6.1 - 7.6)
10) (-)-Quadazocine/Enadoline	6.2 (6.1 - 6.2)-1.7	6.7 (6.6 - 6.8)
11) nor BNI/Enadoline	6.5 (5.9 - 7.0)-1.3	6.6 (5.9 - 7.3)
12) Mecamylamine/(-)-Nicotine	6.6 (6.2 - 6.9)-0.9	-

NIH 10497 *N*-(1*R*-1-Cyclopropyl)ethylmorphine hydrochloride



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - 2.0 (0.6 - 6.6)<sup>a</sup>
- 2) TF vs. M - Inactive at 1, 10 and 30<sup>a</sup>
- 3) PPQ - 0.03 (0.01 - 0.2)<sup>a</sup>
- 4) HP - NT

<sup>a</sup>Reported in NIDA Res. Monog. 95, 1989

Special Tests:

- 1) Naloxone vs ED80 of morphine in TF: 2.98 (1.19 - 7.48)
- 2) Opioid subtype testing
  - a)  $\beta$ -FNA (i.c.v.) vs ED80 of NIH 10497 (s.c.) in TF: Inactive at 1, 10 and 30  $\mu$ g/brain.
  - b) nor-BNI (s.c.) vs ED80 of NIH 10497 (s.c.) in TF: AD50 = 11.17 (2.9 - 42.9) mg/kg.
  - c) Naltrindole (s.c.) vs ED80 of NIH 10497 (s.c.) in TF: 10% at 1, 0% at 10 and 23% at 30 mg/kg.

New Data

Table. Comparison of the antinociceptive effects of three samples of NIH 10497 in the mouse tail-flick test.

Compound No.	Route of Administration	ED50
MCV 4558	s.c.	4.47 (0.51 - 39.08)
NIH10497A	s.c.	2.52 (0.67 - 9.47)
NIH 10497B	s.c.	1.67 (0.31 - 8.96)

MONKEY DATA (Reported in NIDA Monog. 95, 1989)  
(SDS)

NIH 10497 substituted completely for morphine. The drug acted promptly and its duration of action was about 2 hr (see fig). In addition, this drug is slightly less potent than morphine. Many drug-related side effects were seen including body sag, jaw sag, slowing, staring, and salivation. The incidence of drowsiness was more than that observed in morphine-treated controls. [In this context, salivation suggested kappa agonist activity].

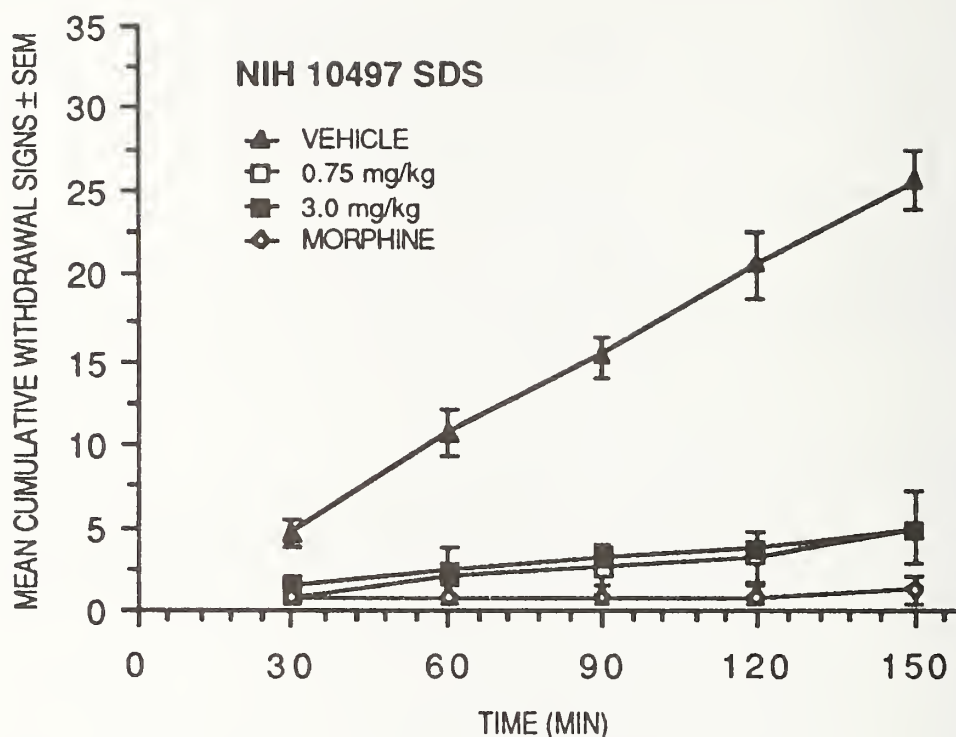


Fig NIH 10497-SDS. Results of study in which single doses of NIH 10497 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: Apparently, NIH 10497 is a selective kappa agonist in both species. Some weak delta-opioid receptor agonist activity was observed.

RAT CONTINUOUS INFUSION ASSAY (PPD) (Reported in NIDA Monog. 95, 1989)

**Primary physical dependence study**

Each rat was randomly allocated a treatment regimen. They were then assigned to a cage on a rack. The 6-day morphine dose regimen that was used by Teiger (1974) was modified by us and shortened to 4 days because studies in our laboratory indicated that the withdrawal syndromes were qualitatively and quantitatively similar. The dose regimen for morphine was 50 mg/kg day on the first day, 100 mg/kg day on day 2, and 200 mg/kg day on days 3 and 4. NIH 10497's low-dose regimen was the same as morphine. The high-dose regimen was double that of the low-dose regimen.

**Physiological and Behavioral Measurements**

During the infusion of vehicle, NIH 10497, or morphine, the rats were weighed and observed daily for 1 hr for overt behavioral signs. Body weight was recorded daily. The sign wet-dog shakes was quantified. Irritability was scored as proposed by Teiger (1974). Scoring for this sign was as follows: 0 (remains tame when touched and on being grasped and lifted); 1 (remains tame when touched and on being grasped and lifted makes only a feeble attempt to wiggle free); 2 (remains tame when touched but when grasped and lifted **claws, bites and** or vocalizes); and, 3 (reacts to initial touch by vocalizing and biting and to attempts to grasp it by rolling over on its back and clawing). All other behavioral signs were simply noted. A trained observer was blind regarding treatment assignments.

### Statistical Analysis

The data from were combined and analyzed. Quantified data was assessed using a repeated measures ANOVA. One factor ANOVA was used to evaluate daily blocks of data. If overall significance was found, Fisher's LSD test was used for post-hoc comparisons. Scored data was analyzed using the nonparametric Kruskal-Wallis one-way ANOVA. Post hoc comparisons of nonparametric data were made using the Mann-Whitney U test. In all cases significance was set at the 95% level. The StatView statistical package (Brainpower, Inc., Agoura Hills, CA) was utilized for these analyses.

### Drugs and Solutions

NIH I0497 was forwarded to us by Andrew Coop of the University of Maryland. Morphine sulfate was purchased from Mallinckrodt, Inc., St. Louis MO. All drugs were dissolved in distilled water and solutions were prepared daily.

### Results

**Body Weight Loss** These results are displayed in Fig. 1. Two-factor repeated measures analysis of variance revealed significant differences among treatment groups ( $F = 5.33$ ,  $P = 0.0083$ ) and days ( $F = 21.048$ ,  $P = 0.001$ ). One factor analysis of body weights at the start of the experiment indicated no significant differences ( $F = 0.172$ ,  $P = 0.9142$ ). One factor ANOVAs for day 3 ( $F = 3.718$ ,  $P = 0.0306$ ), day 4 ( $F = 11.607$ ,  $P = 0.0002$ ), day 5 ( $F = 7.882$ ,  $P = 0.0014$ ), day 6 ( $F = 19.747$ ,  $P = 0.0001$ ), day 7 ( $F = 4.1$ ,  $P = 0.0221$ ), and day 8 ( $F = 25.349$ ,  $P = 0.0001$ ) but not days 1 ( $F = 1.899$ ,  $P = 0.1959$ ), and 2 ( $F = 2.175$ ,  $P = 0.1263$ ) showed significant differences among treatments.

During the infusion of morphine the rats initially showed small increases in body weight during the first 2 days when compared to that of the vehicle controls; the gain was statistically significant on day 1. After morphine was abruptly withdrawn and vehicle substituted, there was a precipitous and significant loss of body weight during the first 24 hr (day 5) followed forty-eight hr later (day 6), by an even greater loss. Although body weights appeared to be recovering during the rest of the experiment, weight loss was still significantly reduced compared to that of vehicle controls.

In sharp contrast with the results obtained with the morphine controls, body weight decreased in a dose-dependent manner in the rats treated with NIH 10497 during its administration and began recovering within 24 hours *after* it was abruptly discontinued. For the high-dose regimen of NIH 10497, weight loss was significant compared to the vehicle control group beginning on day by day 4. It is interesting that although weight loss was still significant compared to the vehicle group at the end of the experiment it was also significantly less than that of the morphine-treated group.

**Wet-dog Shakes** The results are depicted in Fig. 2. When examined for overall differences, 2-factor repeated measures analysis of variance indicated that differences among treatments were significant only for days ( $F = 5.217$ ,  $P = 0.001$ ). One factor ANOVAs for day 6 (48 hr post withdrawal) indicated a significant difference among treatments ( $F = 3$ ,  $P = 0.0578$ ). Comparison of the results of the morphine-treated group with those of the vehicle group was the only comparison among groups that was significant.

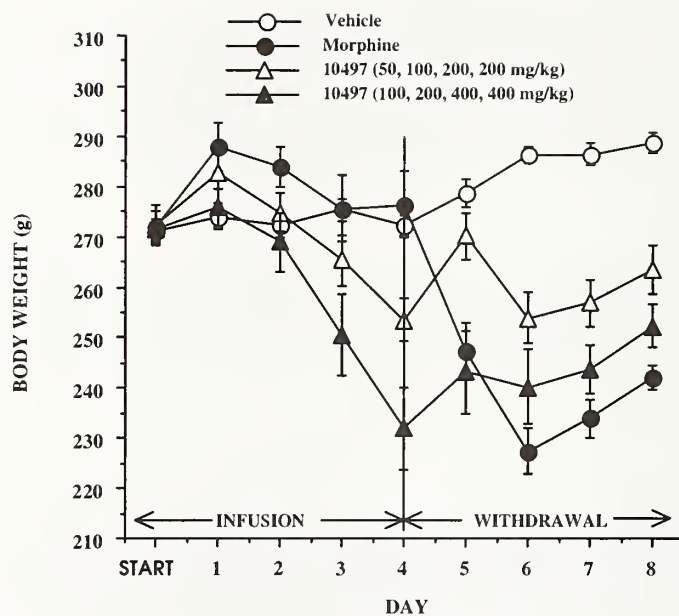


Fig.1. Rat PPD: Body Weight

**Irritability** Fig. 3 displays the results obtained with this sign. The critical value (based on 4 treatments and 3 degrees of freedom) for this experiment using Kruskal-Wallis ANOVA is  $\chi^2_{0.05}(3) = 7.82$  and H for day 5 was 0.357. Post hoc comparisons (Mann-Whitney one-tail test) on this day indicated that only the results of the morphine-treated group showed a significant difference when compared to those of the vehicle group.

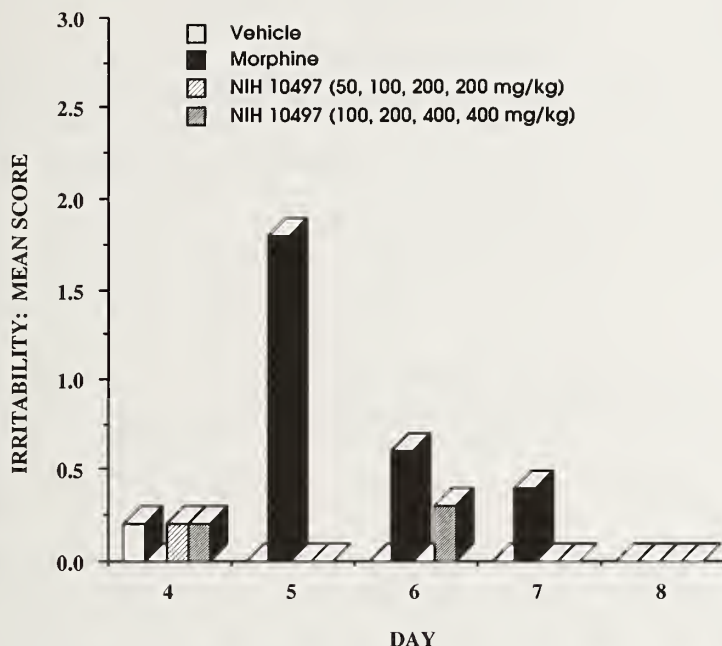


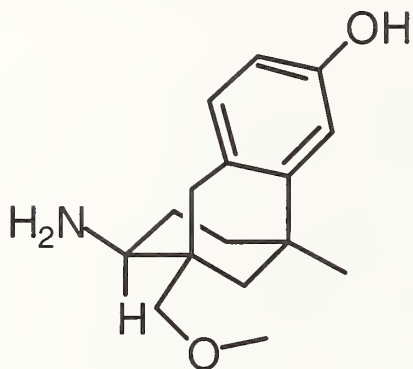
Fig. 3. Rat PPD: Irritability

**Summary**

At dose regimens approximately equal to and double that of morphine sulfate, NIH 10497 did not produce body weight loss, the most reliable index of physical dependence on morphine (Aceto 1990). Neither did it increase the degree of irritability in response to handling, another significant mu-opioid receptor agonist abstinence sign (Himmelsbach *et al.* 1935 and Aceto 1990). Finally, it did not express another important morphine-like abstinence sign designated wet-dog shakes. These results suggest that NIH 10497 is relatively free of mu-opioid induced physical dependence liability.

NIH 10497 has a novel profile of activity. It lacks mu-opioid receptor properties and has selective kappa- and weak delta-opioid receptor effects. Since it substituted for morphine in morphine-dependent monkeys and appears to be free of mu-opioid physical-dependence liability, it may prove to be useful in the pharmacotherapy of heroin-like abuse. However, species difference may have accounted for these results. Finally, because each sample had a different color, they were retested in the tail-flick test. The original sample was yellow and a TLC indicated contamination. Sample A was white and sample B was tan. Samples A and B did not show contamination when examined by TLC.

NIH 10945 ( $\pm$ )-(5*S*,8*S*,9*R*)-8-Amino-3-hydroxy-5,9-methano-9-(methoxymethyl)-5-methylbenzocyclooctene



MOUSE DATA - ED50 OR AD50

(95 % C.L.) (mg/kg or % change)

- 1) TF - 0% at 1, 17% at 10 and 3% at 30<sup>a</sup>
- 2) TF vs M - Inactive at 1, 10 and 30<sup>a</sup>
- 3) PPQ - 3.75 (1.62 - 8.64)<sup>a</sup>
- 4) HP - 25% at 1, 38% at 10 and 38% at 30<sup>a</sup>

<sup>a</sup>Vehicle was 10% hydroxypropyl- $\beta$ -cyclodextrin in water.

New Mouse Data

Special: Naloxone vs ED80 of NIH 10945 in PPQ: AD50 = 2.63 (0.99 - 6.98)

MONKEY DATA  
(SDS)

As shown in the fig below, NIH 10945 reduced the number of withdrawal signs at both doses. Curiously the lower dose appeared more effective in that regard. However, the drug did not substitute completely for morphine in withdrawn morphine-dependent monkeys. Vehicle was 10% hydroxypropyl- $\beta$ -cyclodextrin in sterile water.

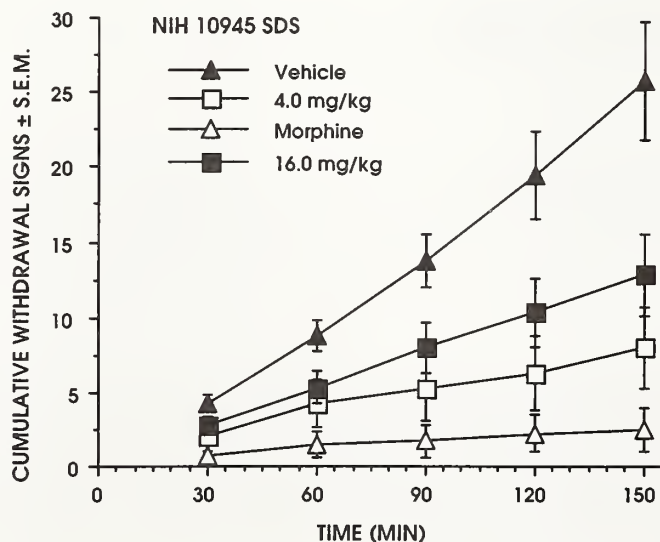
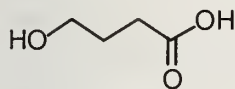


Fig NIH 10945. Results of study in which single doses of NIH 10945 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The compound does not have robust opioid activity. The naloxone AD<sub>50</sub> in the PPQ test suggests weak heterogenous opioid effects. Because of the relatively large AD<sub>50</sub>, most likely the delta-opioid component predominates or the opioid effects are indirect.





Gamma-Hydroxybutyric Acid (GHBA), a precursor and metabolite of gamma-aminobutyric acid, which has been used in Europe, as a general anesthetic and hypnotic, as an aid in childbirth, in the treatment of alcoholism and in anxiety attendant with detoxication from cocaine and amphetamines, depression and other conditions, has also gained popularity as a fashionable recreational drug. Because little is known about its interaction with opioids, this study was initiated. GHBA, per se, (at 30, 60, 80, and 100 mg/kg s.c.) had little effect on the normal reaction time in the tail-flick test. When these doses of GHBA were co-administered with the ED25 of morphine, dose-related synergism was observed. In mice made completely tolerant to morphine antinociceptively (25 mg/kg.s.c., 4 times a day for 4 days), GHBA (60 mg/kg s.c.) in combination with M morphine partially restored antinociception. Naloxone (1 mg/kg s.c.) nearly abolished this effect.

**Table 1. Antinocceptive Effects of GHB in the Mouse Tail Flick and Paraphenylquinone Assays<sup>a</sup>**

Test	Route of Administration	Pretreatment Time	ED50 or AD50 (95% C.L.) (mg/kg or % Change)
Tail-flick	i.v.	20 min	Inactive at 60 and 52% at 120
"	s.c.	20 min	Inactive at 1, 10, 30 and 60
"	p.o.	20 min	Inactive at 60 and 51% at 120
"	p.o.	1 hr	Inactive at 60 and 120
PPQ	i.v.	20 min	30.88 (15.34 - 62.17)

<sup>a</sup>Reported in NIDA Monograph 179, p 363, 1999.

**Table 2. GHB effects in the tail-flick test by the i.c.v. and oral route and orally in combination with 12% alcohol**

Tail-flick	Route of Administration	Treatment	Comment and ED50 or % Change
"	i.c.v (µg/brain)	GHB (mg/kg)	16% at 1, 7% at 10 and 15% at 30 µg/kg
"	p.o.	GHB in 12% alcohol	21% at 30, 32% at 100 mg/kg. At 30 mg/kg mice were ataxic within 5 min of dosing. At 100 mg/kg mice were ataxic and two mice were anesthetized. One regained its righting reflex after 40 min. The other remained anesthetized until it was euthanized.
"	p.o.	12% alcohol controls	Mice were sedated and showed 25% increase in latency

**MONKEY DATA** (Reported in NIDA Monograph 2000, In Press) (SDS)

The results suggest an inverse dose-response relationship (see figure) for GHB regarding attenuation of withdrawal signs in withdrawn morphine-dependent monkeys. Statistical analysis of the data obtained at 150 min, revealed by Kruskal-Wallis one-way analysis of variance, predict highly significant differences among treatment regimens ( $H=23.26, \chi^2 0.005 = 16.75$ ). The Mann-Whitney U test was used to assess between-treatment comparisons. The results indicated that all treatment regimens, except the GHBA 120 mg/kg and GHBA 240 mg/kg, were significantly different from vehicle (at  $U = 6$  or less,  $P = 0.05$  or less). In addition, all GHBA-treated group-withdrawal scores were significantly higher than those in morphine-treated monkeys ( $U = 6$  or less,  $P = 0.01$  or less). Finally, the scores of the low-dose GHB group (7.5 mg/kg) were significantly less than the scores of the highest dose GHBA

group ( $U = 0, P = 0.014$ ), as were those of the 30 mg/kg GHBA group ( $U = 3, P = 0.014$ ). Both the 60 mg/kg-treated GHBA group scores and the 120 mg/kg-treated GHBA group scores were lower than those of the 240 mg/kg-treated GHBA group. However, the differences only approached significance at  $P = 0.056$ .

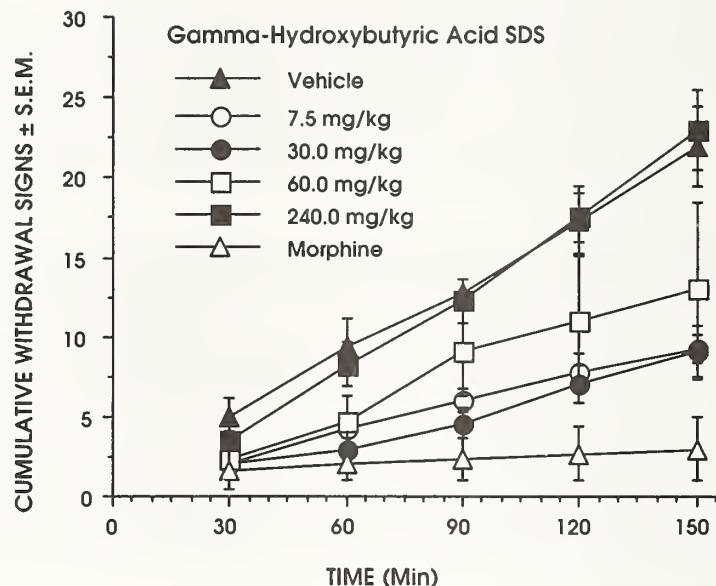


Fig NIH 10947-SDS. Results of study in which single doses of NIH 10947 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: In the mouse and monkey, the results suggest interesting interactions of GHBA with the opioid system which deserve further investigation. In combination with alcohol, GHB produces anesthesia of long duration. Additional studies at lower doses are planned.

NEW DATA

Rat Continuous Infusion Assays

a) Combined four-day primary physical dependence and 2-day substitution for morphine study.

The method has been reported in the literature (Aceto *et al.*, Eur. J. Pharmacol. 307, 2000). Each rat was randomly allocated a treatment regimen. They were then randomly assigned to a cage on a rack. Five treatment regimens consisting of 5-6 rats per regimen were studied. During the 4-day infusion, one group served as the vehicle-vehicle group (8 ml, i.p./24hr.), another group received GHB (50, 100, 200 and 200 mg/kg, i.p./24 hr on days 1 through 4 respectively) and 3 other groups received morphine (same dose regimen as GHB). Then, at the end of 4 days, GHB was abruptly withdrawn and vehicle was substituted in one group. Morphine was also abruptly withdrawn in one of the groups receiving morphine and vehicle was substituted. Finally, 2 dose regimens of GHB (100 or 200 mg/kg, i.p./24hr) were substituted for morphine in the remaining morphine-treated groups. In effect, we conducted a primary physical dependence study and a substitution for morphine study.

Body weight was noted daily, the sign designated wet-dog shakes was quantified. Irritability was scored as follows: 0 (remains tame when touched and on being grasped and lifted); 1 (remains tame when touched and makes only a feeble attempt to wiggle free; 2 (remains tame when touched but when grasped and lifted bites and or vocalizes; and

3 (reacts to initial touch by vocalizing and biting and to attempts to grasp it by rolling over on its back and clawing). All other behavioral signs were simply noted. A trained observer was blind regarding the treatment regimen.

Quantified data were assessed using repeated measures ANOVA. One factor ANOVA was used to evaluate each day. If overall significance was found, the Scheffe test was used for comparisons among means. Scored data were analyzed using Kruskal-Wallis one-way ANOVA (nonparametric test). The Mann-Whitney U test was used for post hoc comparisons. In all cases significance was set at  $p = 0.05$  or less. The Stat View statistical package (Brainpower, Inc., Agoura Hills, CA) was utilized for these analyses. For simplicity sake, the data were separated and illustrated and analyzed according to the components designated abrupt withdrawal (PPD) and substitution for morphine (SM).

1.0 Body Weight

The results of GHB, morphine and vehicle on body weight are illustrated in Fig. 1 below. Repeated measures ANOVA indicated significant differences among days ( $F = 17.149, p = .0001$ ) and for subjects versus treatments ( $F = 8.718, p = .004$ ).

During the infusion, ANOVA revealed significant differences among treatments on days 1 ( $F = 6.398, p = .0116$ ) and 2 ( $F = 11.002, p = .0016$ ). Post hoc analysis showed that the morphine-vehicle controls increased their weight significantly compared to either the vehicle-vehicle group or the GHB-vehicle group. It should be noted that with this dose regimen morphine controls typically behave this way. On days 3 and 4 the F values were not significant indicating no significant differences in body weight among the treatment groups.

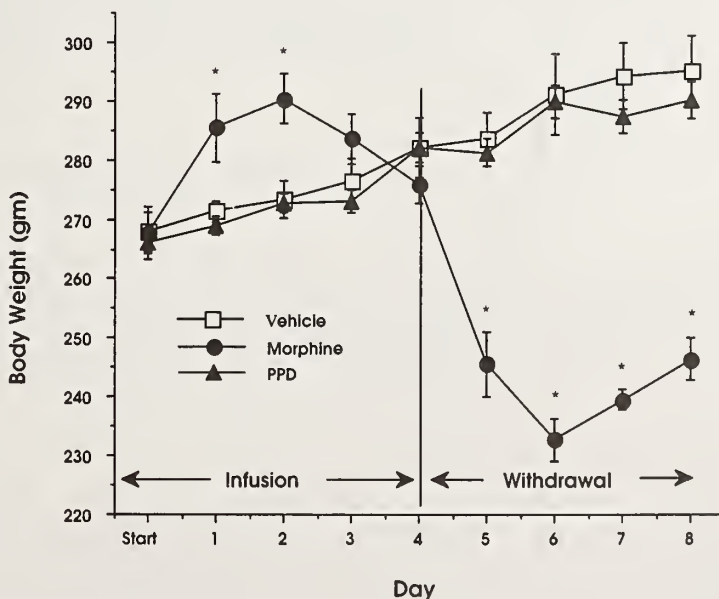


Fig. 1. PPD: Depicted results of the primary physical dependence study of GHB and morphine. \*Significant at  $p = 0.05$ .

Dramatic changes occurred during the withdrawal. ANOVAs for days 5 and 6 indicated highly significant Fs ( $F = 25.632, p = .0001$  and  $F = 51.344, p = .0001$  respectively). However, post hoc comparisons indicated that only the rats in the morphine-vehicle group lost significant body weight compared to the vehicle-vehicle-treated group. ANOVAs for the final 2 days of the experiment also indicated significant differences among the treatment groups ( $F$

= 62.818,  $p = .0001$  for day 7 and  $F = 37.519$ ,  $p = .0001$  for day 8). Again, compared to the vehicle-vehicle control group, only the morphine-vehicle-treated group showed a significant loss of body weight. However, the morphine group appeared to be recovering. The vehicle-vehicle and GHB-vehicle groups steadily gained weight throughout the study.

Body weight of the SM study is illustrated in Fig 2. Repeated measures ANOVA indicated significant differences among treatments ( $F = 7.683$ ,  $p = .0021$ ) and days ( $F = 177.627$ ,  $p = .0001$ ). ANOVA by day revealed no significant values at the start of the experiment ( $F = .002$ ,  $p = .9999$ ) and on day 1 ( $F = 2.357$ ,  $p = .1058$ ). On day 2 significant difference were evident among all treatments ( $F = 5.539$ ,  $p = .0071$ ); the morphine groups, had significantly increased weight compared to the vehicle group. However, ANOVA for days 3 and 4 showed that all groups were now equivalent regarding weight. ( $F = .576$ ,  $p = .6381$  and  $F = .884$ ,  $p = .468$ , respectively). Twenty-four and 48 hr (days 5 and 6) after morphine was withdrawn, neither vehicle nor GHB substitution (100 or 200 mg/kg/24 hr, prevented severe loss of body weight ( $F = 25.948$ ,  $p = .0001$  for day 5 and  $F = 42.633$ ,  $p = .0001$ ). Post hoc comparison with the vehicle-vehicle group confirmed this observation. Thus, GHB did not substitute for morphine. Although body weight remained depressed throughout the remainder of the experiment ( $F = 28.474$ ,  $p = .0001$  on day 7 and  $F = 24.963$ ,  $p = .0001$  on day 8 and confirmed by post hoc analyses) recovery was evident.

(Kruskal-Wallis one-way analysis of variance revealed significant differences among treatment regimens ( $H = 14.505$  for day 5 and  $14.667$  for day 6. The critical value of  $\chi^2_{0.05(2)} = 5.99$ . Post hoc comparisons using the Mann-Whitney U test confirmed that only the morphine-vehicle group had significantly elevated scores on days 5 and 6 (24 and 48 hr post withdrawal).

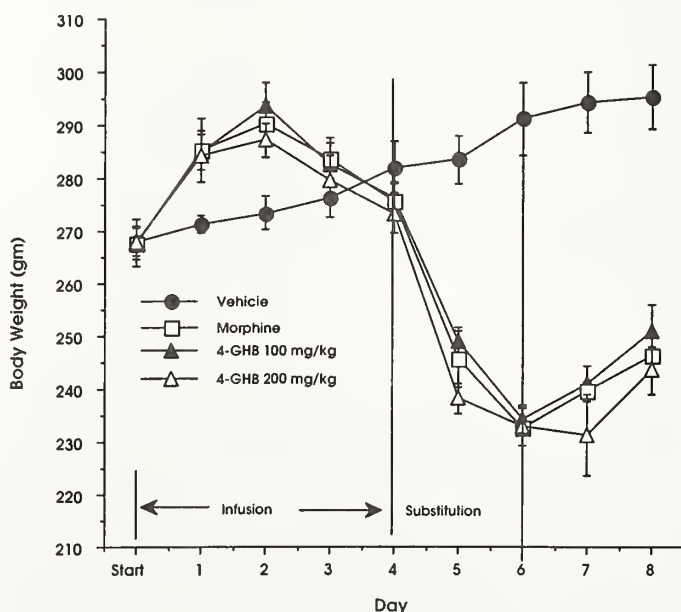


Fig. 2. Illustration of the effects of GHB on body weight either after abrupt withdrawal (end of day 4 to day 8) or when it was substituted for substituted for morphine (days 5 and 6).

## NIH 10947 (Continued)

### 2.0 Irritability

Irritability associated with morphine withdrawal was not blocked or attenuated after substituting either of the two dose regimens of GHB or vehicle for morphine (SM). ( $H = 11.236$  for day 5,  $19.583$  for day 6, and  $20.778$  for day 7,  $\chi^2_{0.05(3)} = 7.82$ ). Post hoc analyses indicated that all the rats receiving morphine had elevated irritability scores. Thus, GHB did not substitute for morphine.

### 3.0 Wet-Dog Shakes

Before abrupt withdrawal of GHB (day 4), ANOVA indicated no significant differences among treatments ( $F < 1$ ). After withdrawal,  $F$  approached significance on day 5, ( $F = 2.426$ ,  $p = .1272$ ) and was significant on day 6 ( $F = 11.114$ ,  $p = .0015$ ),  $p = .0421$ . The Scheffe test confirmed that the morphine-vehicle group displayed a significantly increased number of wet-dog shakes vis a vis the vehicle-vehicle group.

The incidence of wet-dog shakes was not significantly before GHB was substituted for morphine (day 4,  $F < 1$ ). After substitution (day 5), ANOVA did not suggest significant differences among treatments ( $F = 1.716$ ,  $p = .1995$ ). However, at 48 hr after substitution (day 6) ANOVA indicated significant differences among treatments ( $F = 3.351$ ,  $p = .0421$ ). Unfortunately, Scheffe's test did not identify the treatment group(s) with increased scores.

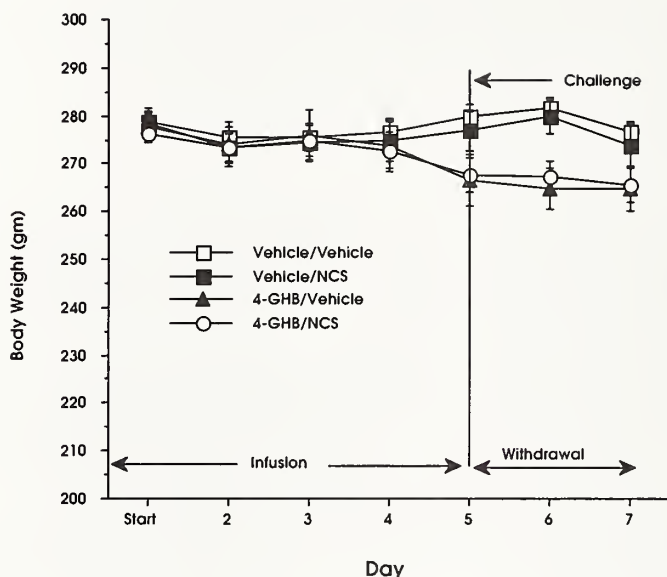
#### B. Combined high-dose 5-day primary physical dependence (PPD) and precipitated-withdrawal study (PPt-W)

Four groups of 6-8 rats per group were prepared for infusion. Then, one group received vehicle (8 ml/24hr) for 5 days and then challenged with a single injection of vehicle (i.p.) Another group received vehicle by infusion as above and then challenged with a single injection of the GHB antagonist NCS-382 (50 mg/kg i.p.). Two groups each received a dose regimen of 600, 1200, 2400, 4800 and 4800 mg/kg, i.p./24hr of GHB for 5 days, in that order. Next, one group of GHB-treated rats was given a single dose of NCS-382 (i.p.) and the other was given vehicle (i.p.) The same experimental design outlined above was observed. In addition, behavioral observations were recorded 1 hr before and 1 hr after challenge with vehicle or NCS-382.

### Body Weight

Repeated measures ANOVA indicated significant differences among days ( $F = 6.869$ ,  $p = .0001$ ) as well as a significant interaction days and body weight ( $F = 3.916$ ,  $p = .0001$ ).

The body-weight results are displayed in Fig. 3 below. As can be seen in the figure, the body weights of all the body weight of the treatment groups remained essentially the same until day 5: no significant changes were calculated: at the start, of the experiment ( $F = .208$ ,  $p = .8901$ ) or on days 1, 2, and 3 ( $F = .101$ ,  $p = .9588$ ,  $F = .053$ ,  $p = .9836$ , and  $F = .471$ ,  $p = .7051$ , respectively). On day 4, statistical significance was achieved. ANOVA revealed that  $F = 2.564$ ,  $p = .0783$ .



**Fig. 3. Body weight changes before and after abrupt and precipitated withdrawal of GHB.**

Apropos abrupt withdrawal, there is no evidence that this occurred since weight loss commenced before GHB was withdrawn. F was not significant ( $F = 1.973, p = .449$ ). Concerning precipitated withdrawal, a statistically significant weight loss was evident on day 5 ( $F = 5.432, p = .0054$ ). However, this weight loss was probably due to GHB since it occurred before the NCS-382 challenge.

**2.0 Irritability**

There was no evidence that the sign irritability was expressed either after abrupt withdrawal of GHB or after challenge with NCS-3382. All the scores were either 0 or 1.

**3.0 Wet-Dog Shakes**

Repeated measures analysis of the abrupt withdrawal and precipitated withdrawal data indicated no significant differences regarding the intervals 1 hr pre and post challenge, and 24 hr post challenge with regard to the sign wet-dog shakes (A) ( $F = 2.314, p = .1014$ ) intervals (B) ( $F = 2.41, p = .1006$ ), or their interaction (AB) ( $F = 1.393, p = .2368$ ). Also, one-way ANOVA for intervals revealed no significant differences among treatments.

**Summary and Conclusions**

GHB was given continuously to rats using two dose regimens of 50, 100, 200 and 200 mg/kg /24 hrs for 4 days respectively and 600, 1200, 2400, 4800 and 4800 mg/kg/24 hr for 5 days, respectively.

At the lower dose regimen, there was no effect on body weight either during its administration or after its abrupt withdrawal. Typical withdrawal signs designated irritability and wet-dog shakes were not observed. In addition, at doses of 200 mg/ kg/24 hr for 2 days GHB did not substitute for morphine in withdrawn dependent rats.

At the much higher dose regimen, GHB did not express signs of physical dependence either when it was abruptly discontinued or after challenge with a purported antagonist (NCS-382). A non statistically significant trend in body-weight loss began during its administration. After abrupt withdrawal or after challenge with NCS-382 the decline

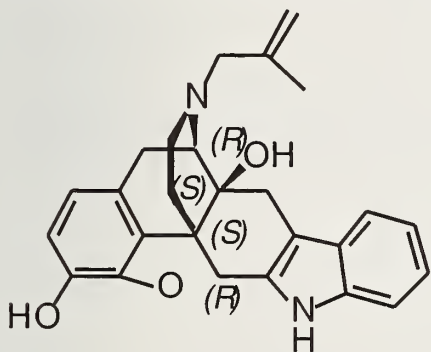
plateaued. This was the only overt sign of drug effect. The withdrawal signs irritability and wet-dog shakes were not noted.

We conclude that the physical dependence liability of GHB is very low.

#### General Comment

GHB interacts with the opioid system producing antinociceptive synergy with morphine and reversal of tolerance to morphine in mice. Depending on the dose, it attenuates withdrawal signs in morphine-dependent monkeys. Finally, it has very low toxicity and physical dependence capacity in rats. GHB may be useful in man in the pharmacotherapy of pain and dependence.

#### NIH 10978 *N*-(3-Methylallyl)noroxymorphindole



#### MOUSE DATA - ED50 OR AD50 (95 % C.L.) (mg/kg or % change)

- 1) TF – 1% at 1, 0% at 10 and 3% at 30<sup>a</sup>
- 2) TF vs. M – 3% at 1, 31% at 10, 51% at 30 and 46% at 60<sup>a</sup>
- 3) PPQ – Inactive at 1, 7% at 10 and 30% at 30<sup>a</sup>
- 4) HP – Inactive at 1, 10 and 30<sup>a</sup>

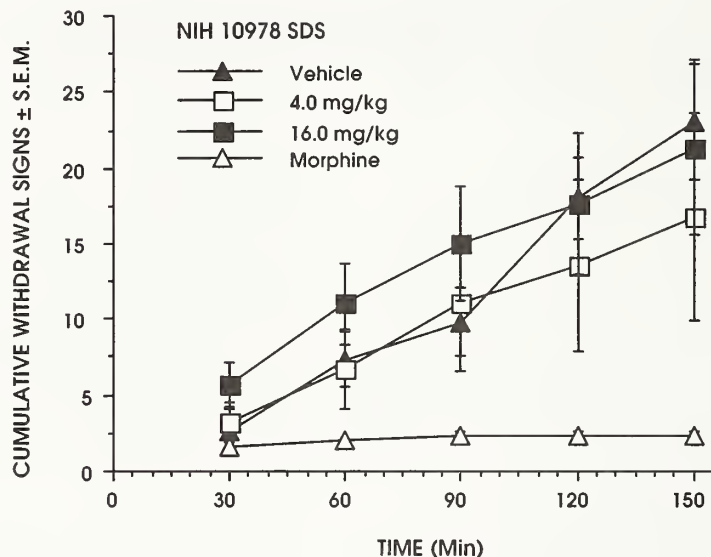
<sup>a</sup>Vehicle was 30% hydroxypropyl- $\beta$ -cyclodextrin in water.

#### MONKEY DATA (SDS)

The accompanying illustration indicates lack of significant effects by NIH 10978 in withdrawn morphine-dependent monkeys. It neither substitutes for morphine nor exacerbates withdrawal. Vehicle was 30% hydroxypropyl- $\beta$ -cyclodextrin in water. Perhaps the drug was not sufficiently absorbed or had a delayed onset of action.

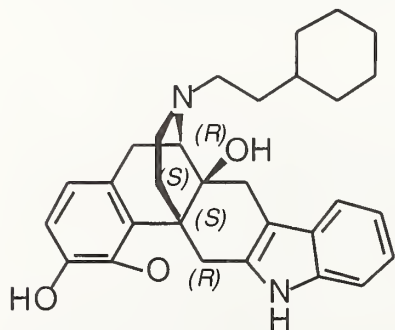
NIH 10978 (Continued)

Fig NIH 10978-SDS. Results of study in which single doses of NIH 10978 were substituted for morphine in morphine-dependent monkeys in withdrawal.



Comment: Studies in the mouse suggest weak mu-opioid receptor antagonist properties. Some non dose-related antinociceptive activity was observed in the PPQ test. Data from the substitution for morphine studies in monkeys suggest that NIH 10978 neither substitutes for morphine nor exacerbates withdrawal.

NIH 10979 *N*-Cyclohexylethylmorphindole.HCl



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - 3.86 (2.66 - 5.59)<sup>a,b</sup>
- 2) TF vs. M - Inactive at 1, 10 and 30
- 3) PPQ - 0.39 (0.17 - 0.86)
- 4) HP - 2.42 (1.57 - 3.73)

<sup>a</sup>Straub tails and increased locomotor activity at 6.  
<sup>b</sup>Increased locomotor activity at 4.

Special Test: Naloxone vs ED80 of NIH 10979 in TF test: AD50 = 0.1 (0.07 - 0.6)

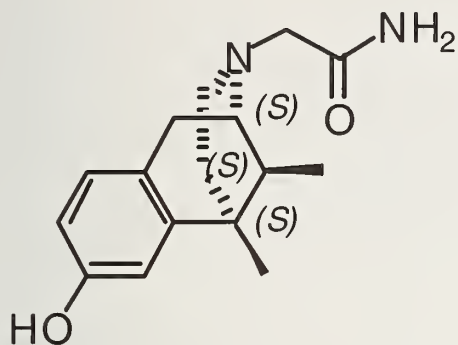
MONKEY DATA  
(SDS)

Not Tested.

Comment: The antinociceptive and behavioral profiles suggest opioid involvement. The high AD50 value with naloxone implicates multiple opioid-receptor agonist mechanisms.



NIH 10992 (+)-(1*S*,5*S*,9*S*)-2-Acetamido-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - Inactive at 1, 10 and 30<sup>a</sup>
  - 2) TF vs. M - 0% at 1, 5% at 10 and 9% at 30<sup>a</sup>
  - 3) PPQ - 4.05 (1.82 - 9.05)<sup>a</sup>
  - 4) HP - Inactive at 1, 10 and 30.
- <sup>a</sup>Vehicle was 5% hydroxypropyl- $\beta$ -cyclodextrin in water.

MONKEY DATA  
(SDS)

The data are not indicative of activity at 4 and 16 mg/kg. Thus, NIH 10992 (see figure) neither substituted for morphine nor exacerbated withdrawal. Vehicle was dilute HCl in water.

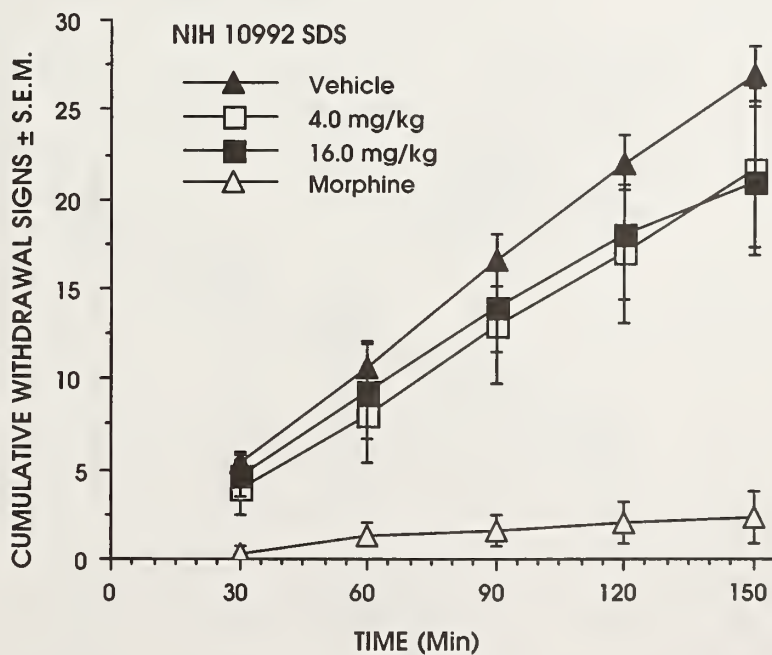
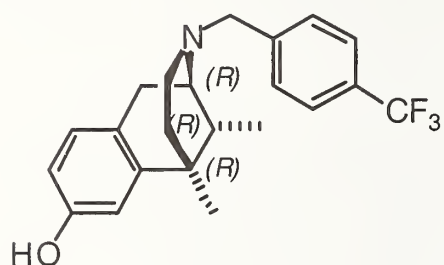


Fig NIH 10992-SDS. Results of study in which single doses of NIH 10992 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The results in mice and monkeys are not suggestive of remarkable opioid properties.

NIH 10994 (-)-(1R,5R,9R)-5,9-Dimethyl-2'-hydroxy-2-(4-trifluoromethylbenzyl)-6,7-benzomorphan .oxalate



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF – 3% at 1, 0% at 10 and 8% at 30<sup>a,b</sup>
  - 2) TF vs. M – Inactive at 1, 10 and 30<sup>a,b</sup>
  - 3) PPQ – 27.40 (11.30 - 66.41)<sup>a,b</sup>
  - 4) HP – Inactive at 1 and 10, 13% at 30<sup>a,b</sup>
- <sup>a</sup>Vehicle was 5% hydroxypropyl- $\beta$ -cyclodextrin in water.

MONKEY DATA  
(SDS)

At doses of 4 and 16 mg/kg, NIH 10994 neither substituted for morphine nor exacerbated withdrawal. At the high dose, jaw sag was observed in one monkey. Drug supply was exhausted. Vehicle was 10% hydroxypropyl- $\beta$ -cyclodextrin in water.

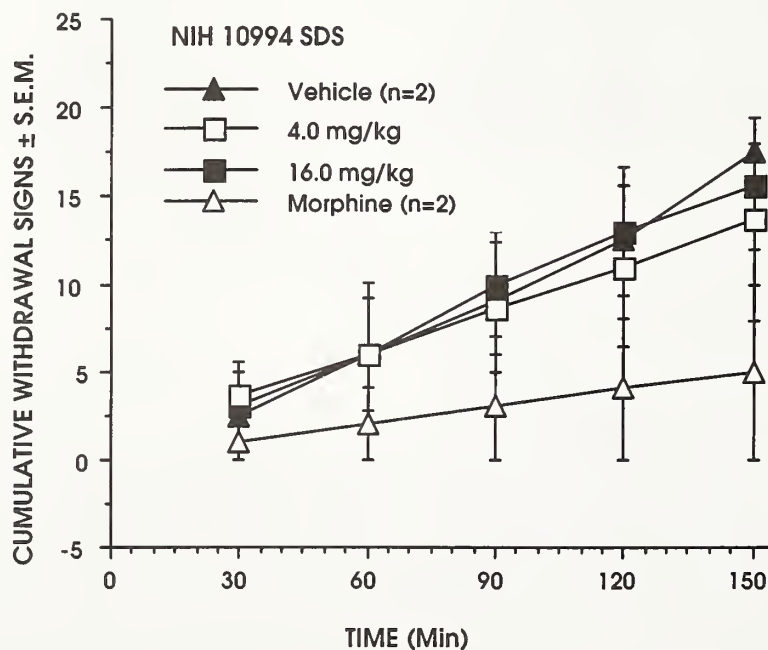
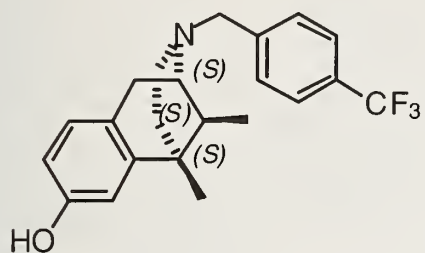


Fig NIH 10994-SDS. Results of study in which single doses of NIH 10994 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The results do not portend mu-opioid agonist or antagonist activity. Very weak antinociceptive action was noted in the PPQ test in mice.

**NIH 10995** (+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2'-hydroxy-2-(4-trifluoromethylbenzyl)-6,7-benzomorphan .oxalate



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF – 3% at 1, 1% at 10 and 7% at 30<sup>a,b</sup>
- 2) TF vs. M – Inactive at 1, 10 and 30<sup>a,b</sup>
- 3) PPQ – 32.81 (16.44 - 65.45)<sup>a,b</sup>
- 4) HP – Inactive at 1 and 10, 25% at 30<sup>a,b</sup>

<sup>a</sup>Vehicle was 5% hydroxypropyl- $\beta$ -cyclodextrin in water.

MONKEY DATA  
(SDS)

The results shown in the figure suggest an inverse dose response, i.e., the low dose attenuated withdrawal behavior more than the high dose. Most of the attenuation of withdrawal signs were associated with the reduction in the number of signs designated vocalization when the abdomen was palpated and decreased abdominal rigidity.

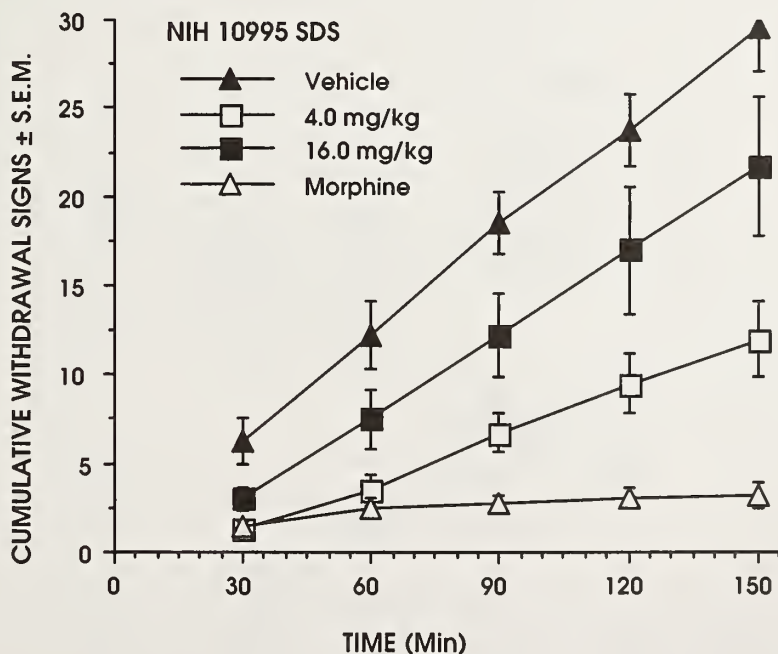
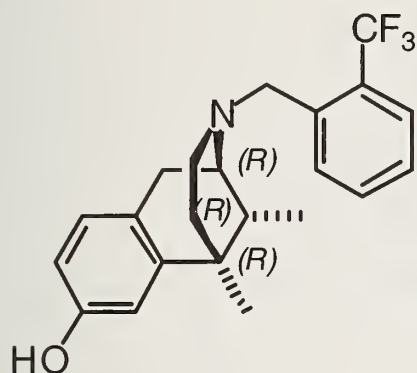


Fig NIH 10995-SDS. Results of study in which single doses of NIH 10995 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: Evidence for mu-opioid agonist activity is unimpressive.





MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF – Inactive at 1, 10 and 30
- 2) TF vs. M – Inactive at 1, 10 and 30
- 3) PPQ – 0% at 1, 8% at 10 and 9% at 30
- 4) HP – Inactive at 1, 10 and 30

Vehicle was 5% hydroxypropyl- $\beta$ -cyclodextrin in water.

MONKEY DATA  
(SDS)

As illustrated in the accompanying figure, NIH 11004 did not substitute for morphine, attenuate or exacerbate withdrawal behavior. Vehicle was 10% hydroxypropyl- $\beta$ -cyclodextrin in water.

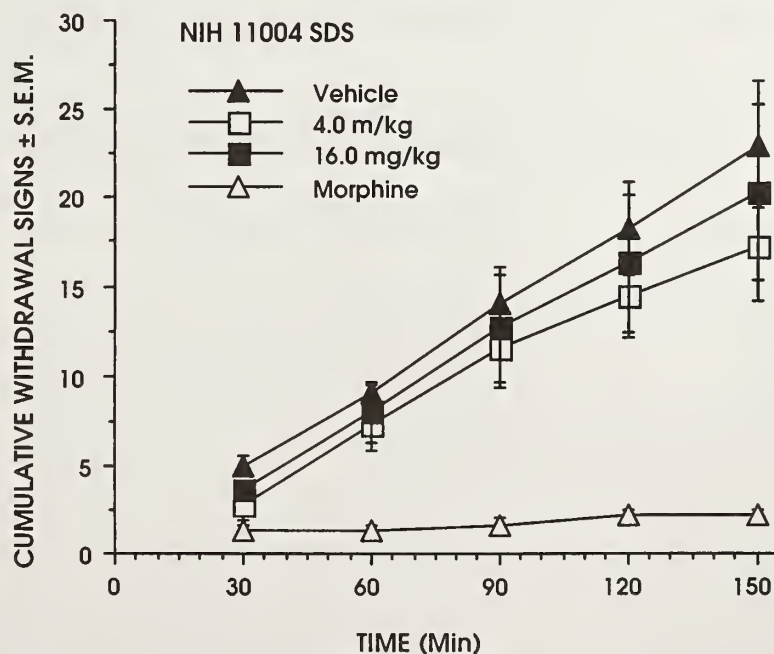
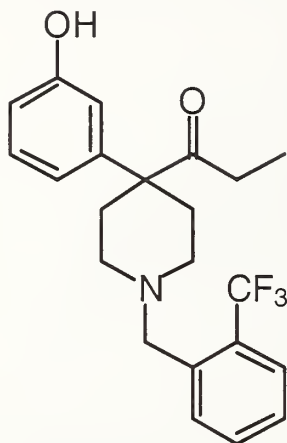


Fig NIH 11004-SDS. Results of study in which single doses of NIH 11004 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: Evidently NIH 11004 is devoid of opioid properties in these bioassays.

**NIH 11005** 4-(3-hydroxyphenyl)-4-(1-oxo-propyl)-1-(2-trifluoromethylbenzyl)piperidine.HCl



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF – Inactive at 1, 10 and 30
- 2) TF vs. M – Inactive at 1, 10 and 30
- 3) PPQ – 14% at 10, 49% at 30 and 51% at 60
- 4) HP – 0% at 1 and 10, 13% at 30

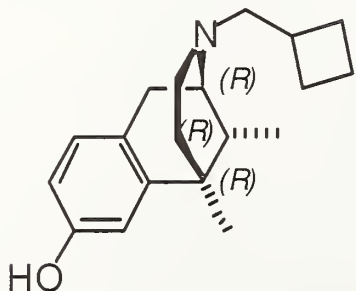
Vehicle: 5% hydroxypropyl- $\beta$ -cyclodextrin

MONKEY DATA  
(SDS)

Due to limited supplies, only the preliminary study was conducted. Doses of 1, 2, 4 and 8 mg/kg were given at 15 min intervals, respectively. The cumulative dose of 15 mg/kg was without effect. Vehicle was 10% hydroxypropyl- $\beta$ -cyclodextrin in water.

Comment: NIH 11005 has weak, if any, antinociceptive properties in mice. The data do not indicate significant mu-opioid receptor activity.

**NIH 11006** (-)-(1*R*,5*R*,9*R*)-2-Cyclobutylmethyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.HCl



MOUSE DATA - ED50 OR AD50  
(95% C.L.) (mg/kg or % change)

- 1) TF – 0.26 (0.07 - 0.88)
- 2) TF vs. M – Inactive at 1, 10 and 30
- 3) PPQ – 0.04 (0.02 - 0.08)
- 4) HP – 0.12 (0.044 - 0.31)

Special Test: Naloxone AD50 vs ED80 of NIH 11006 in TF = 0.84 (0.35 - 2.0) mg/kg.

MONKEY DATA  
(SDS)

NIH 11006 completely alleviated withdrawal signs at 0.25 mg/kg. However, accompanying this reduction of withdrawal signs were a number of other signs designated ataxia, jaw sag, salivation, tremor and eyelid ptosis. Salivation, in this context, suggests kappa-opioid receptor activity.

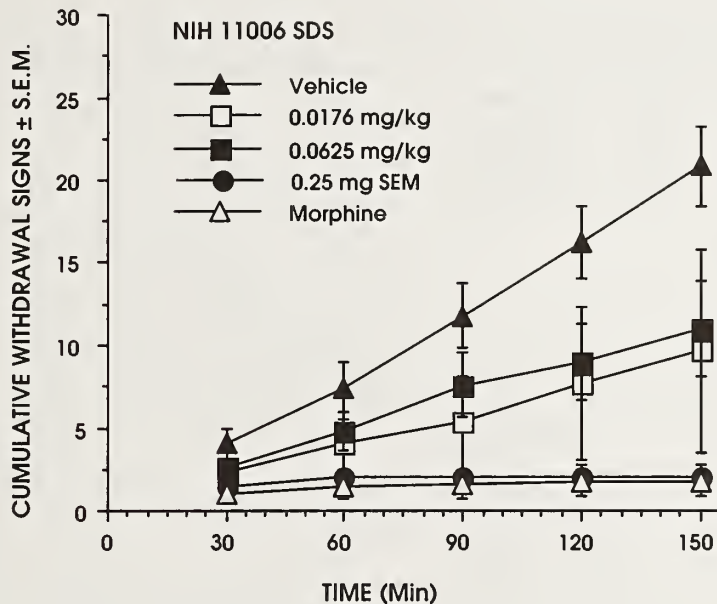
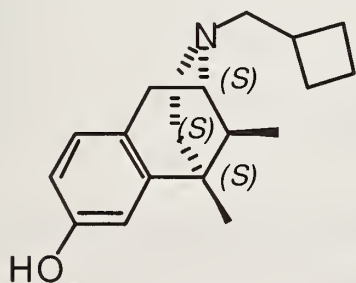


Fig NIH 11006 SDS. Results of study in which single doses of NIH 11006 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The relatively high naloxone AD50 in the mouse tail-flick test and the results in the monkeys suggest heterogeneous opioid activity with a strong kappa-opioid subtype component.

**NIH 11007 (+)-(1*S*,5*S*,9*S*)-2-Cyclobutylmethyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan. HCl**



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - Inactive at 1, 10 and 30
- 2) TF vs. M - 0% at 1, 12% at 10 and 19% at 30
- 3) PPQ - 14% at 1, 3% at 10 and 14% at 30
- 4) HP - Inactive at 1, 10 and 30

MONKEY DATA  
(SDS)

These results should be considered as preliminary since the drug supply was exhausted before the experiment was completed. Attenuation of withdrawal was accompanied by ataxia and slowing at the high dose. Other behavioral signs observed at the 16 mg/kg included walking in circles, spinning while sitting and staggering during first 1/2 hour.

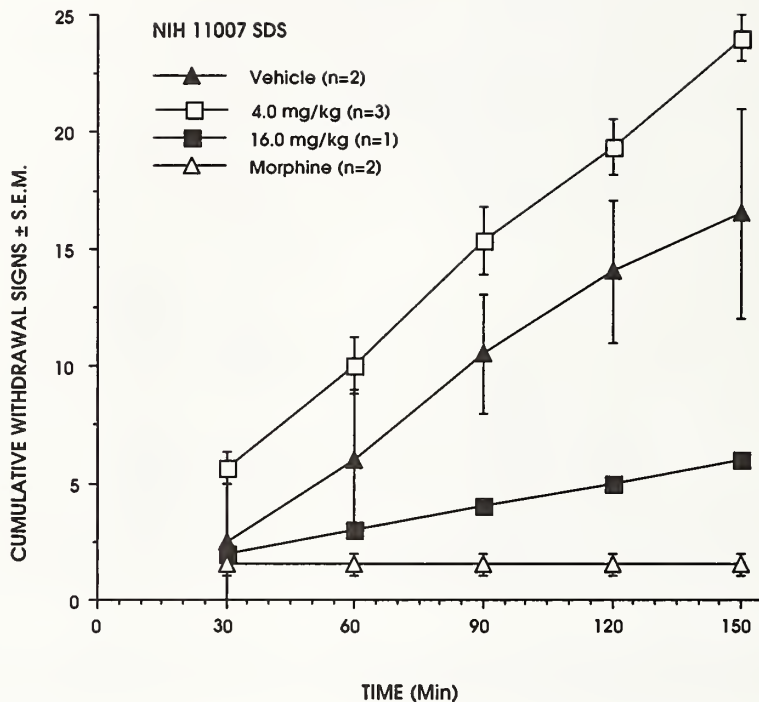
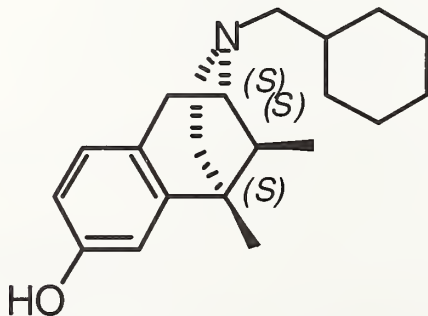


Fig NIH 11007-SDS. Results of study in which single doses of NIH 11007 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: NIH 11007 appears to be devoid of mu-opioid properties. However, CNS effects are prominent.

NIH 11011 (+)-(1*S*,5*S*,9*S*)-2-Cyclohexylmethyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan. HCl



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - Inactive at 1, 10 and 30
- 2) TF vs. M - 20% at 1, 1% at 10 and 7% at 30
- 3) PPQ - 17.57 (5.91 - 52.26)
- 4) HP - 0% at 1 and 10, 13% at 30



MONKEY DATA  
(SDS)

Drug supply was exhausted. A complete evaluation of NIH 11011 was precluded. Attenuation of withdrawal signs at 16 mg/kg was accompanied by jaw sag and ataxia. These behavioral signs and salivation were also noted at the lower dose.

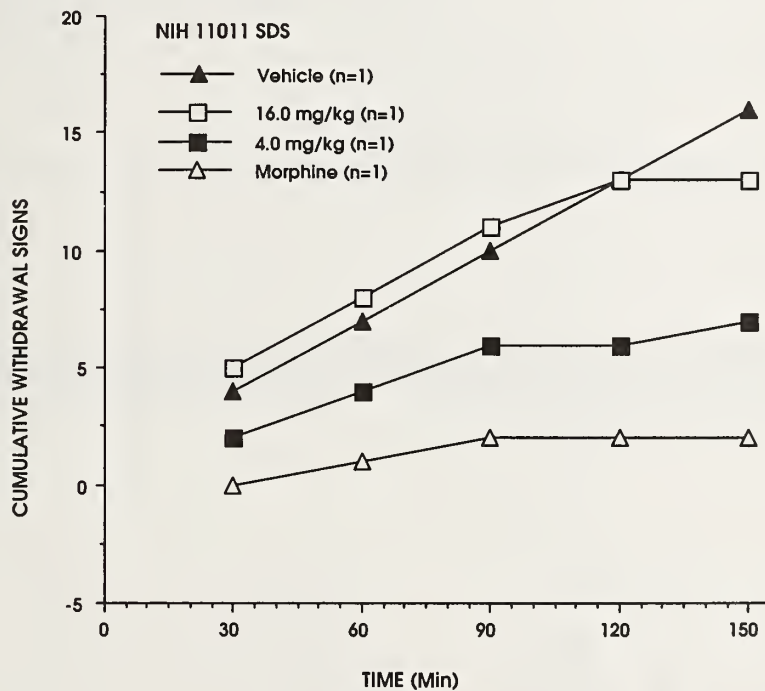
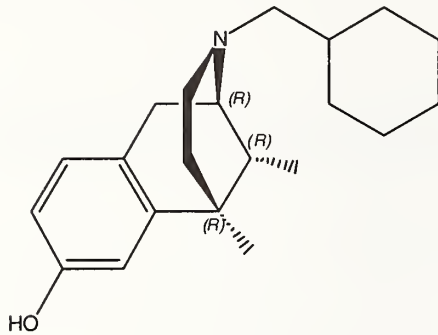


Fig NIH 11011-SDS. Results of study in which single doses of NIH 11011 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The profile of activity does not suggest mu opioid-receptor properties.

NIH 11012 (-)-(1R,5R,9R)-2-Cyclohexylmethyl-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan. HCl



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - 3% at 1 and 10, 0% at 30<sup>a</sup>
- 2) TF vs. M - 3.70 (1.02 - 13.45)<sup>a</sup>
- 3) PPQ - 14.62 (3.12 - 68.3)<sup>a,b</sup>
- 4) HP - 0% at 1 and 10, 13% at 30<sup>a</sup>

<sup>a</sup>Vehicle was 4% Tween 80 in water.

<sup>b</sup>One mouse at 60 mg/kg was very lethargic and one mouse had convulsions that lasted to end of experiment.

<sup>c</sup>Vehicle was 5% hydroxypropyl- $\beta$ -cyclodextrin in water.

Special Test:

- 5) Naltrindole (s.c.), (30 min pretreatment) vs NIH 11012 ED80 in the PPQ test: 15% at 1, and 13% at 10 and 30<sup>c</sup> mg/kg.

MONKEY DATA  
(SDS)

At doses of 0.75 and 3.0 mg/kg, NIH 11012 did not substitute for morphine or exacerbate withdrawal. Jaw sag was noted at 3 mg/kg. One monkey who received 12 mg/kg had tremors followed by convulsions. Pentobarbital (30 mg/kg, i.p.) effectively terminated the convulsions.

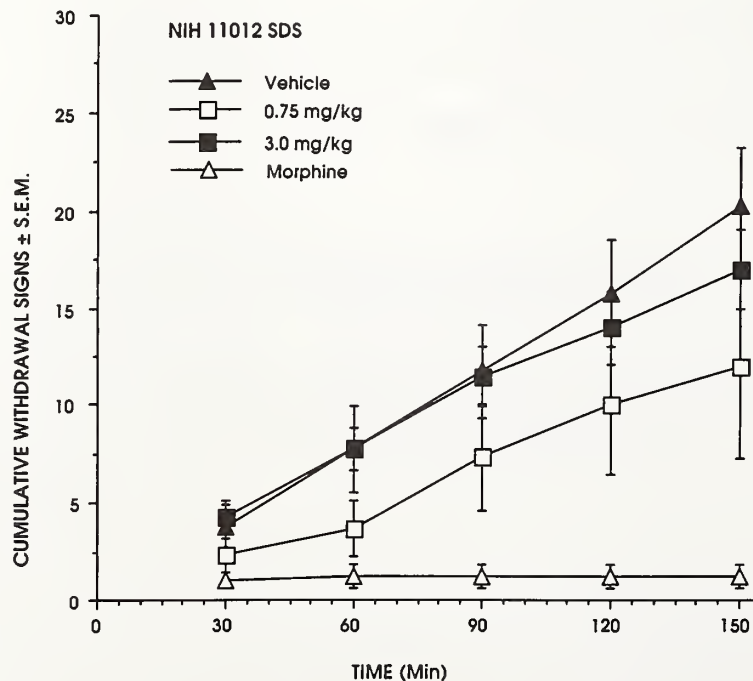
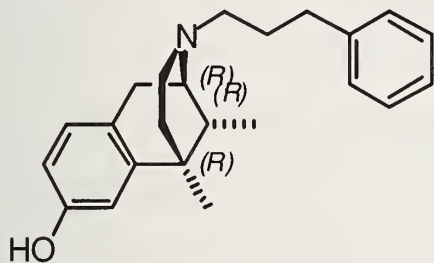


Fig NIH 11012-SDS. Results of study in which single doses of NIH 11012 were substituted for morphine in morphine-dependent monkeys in withdrawal.

## NIH 11012 (Continued)

Comment: The results suggest that NIH 11012 has weak mu-opioid antagonist properties. Some antinociception was noted in the PPQ test which was not delta-opioid receptor related. The drug also produced convulsions in both species.

## NIH 11013 (-)-(1R,5R,9R)-2-(3-Phenylpropyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan. HCl



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - 8.86 (3.64 - 21.60)
- 2) TF vs. M - 14% at 1, and 0% at 10 and 30
- 3) PPQ - 4.59 (2.9 - 7.2)
- 4) HP - 9.63 (2.92 - 31.81)

Vehicle: 2 drops lactic acid in water.

## MONKEY DATA (SDS)

As shown in the figure, NIH 11013 partly substituted for morphine at the high dose. At this dose the signs designated ataxia, jaw sag, slowing and eyelid ptosis were noted. Onset of action was prompt; however less than morphine's. In the preliminary study in one monkey, an accumulative dose of 7 mg/kg was associated with labored respiration.

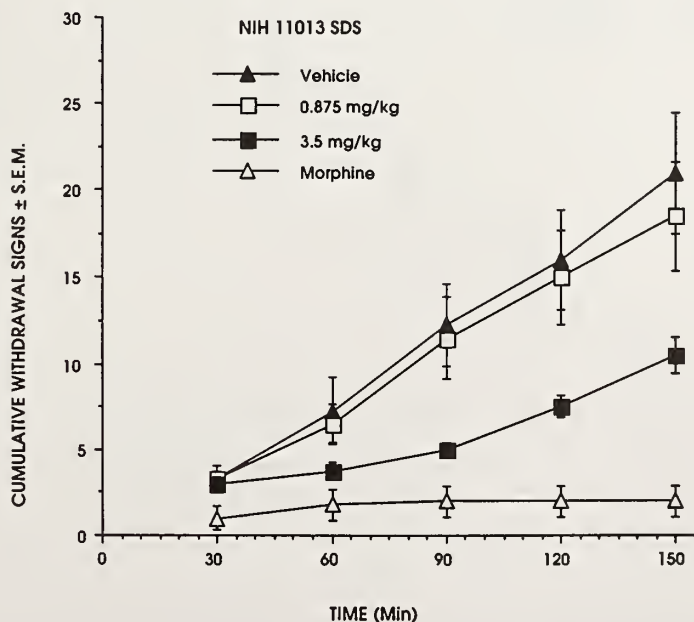
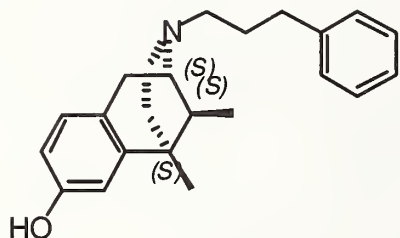


Fig NIH 11013-SDS. Results of study in which single doses of NIH 11013 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The profile of activity suggests heterogenous opioid and/or central nervous system effects.

NIH 11014 (+)-(1S,5S,9S)-2-(3-Phenylpropyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan. HCl



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - 19.14 (11.57 - 31.66)<sup>a</sup>
- 2) TF vs. M - Inactive at 1, 10 and 30
- 3) PPQ - 1.42 (0.39 - 5.18)<sup>b</sup>
- 4) HP - 22.06 (16.81 - 28.94)<sup>c</sup>

<sup>a</sup> At 30 eyelid ptosis and immobility. Mice didn't move when touched.

<sup>b</sup> At 30 decreased locomotor activity (mice nearly immobile). Eyelid ptosis was also noted.

<sup>c</sup> At 30 decreased locomotor activity. Eyelid ptosis was noted at 15, 20 and 30.

MONKEY DATA  
(SDS)

NIH 11014 produced a very weak non dose-related attenuation of withdrawal signs (see figure). At the high dose, jaw sag, salivation and eyelid ptosis were noted in one monkey. Drug supply was exhausted (n=2).

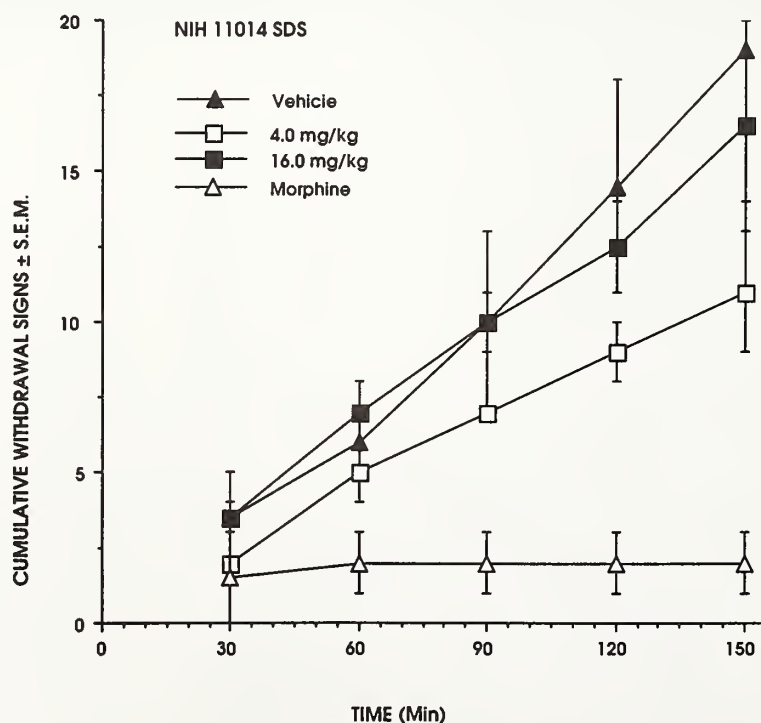
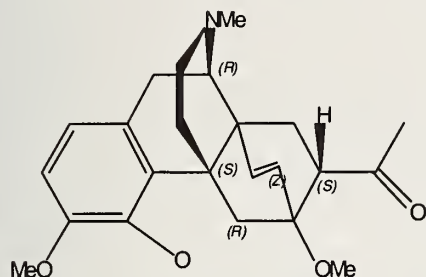


Fig NIH 11014 SDS. Results of study in which single doses of NIH 11014 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: Additional supplies might help clarify the profile of this drug. It appears to be a weak opioid compound and scant evidence suggests kappa-opioid activity and/or other CNS effects.

NIH 11015 (10631) Thevinone.oxalate



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - 5.65 (2.89 - 11.08)
- 2) TF vs. M - 2% at 1, 13% at 10 and 0% at 30
- 3) PPQ - 2.36 (1.02 - 5.45)
- 4) HP - 4.49 (1.61 - 12.54)

Special Tests: Naloxone (s.c.) vs ED80 of NIH11015 (s.c.) in TF: 0.02 (0.008 - 0.52)

Opioid subtype testing

- a)  $\beta$ -FNA (i.c.v.) vs ED80 of NIH 11015 (s.c.) in TF: 0% at 0.1, 28% at 0.03, 26% at 0.1, 62% at 0.3, 72% at 1, 37% at 3, 65% at 10 and 69% at 30.
- b) Nor-BNI (s.c.) vs ED80 of NIH 11015 (s.c.) in TF: 7% at 1, 13% at 3, 47% at 10, 56% at 30 and 23% at 60.
- c) Naltrindole (s.c.) vs ED80 of NIH 11015 (s.c.) in TF: 6% at 3, 43% at 10, 47% at 10 and 36% at 30.

MONKEY DATA  
(SDS)

Doses of 2 and 8 mg/kg completely substituted for morphine in morphine-dependent monkeys in spontaneous withdrawal. At the high dose, the signs scratching, ataxia, jaw and body sag and eyelid ptosis were observed. The drug acts promptly and duration of action was shorter than that of morphine. NIH 11015 appears to be as potent as morphine.

NIH 11015 (Continued)

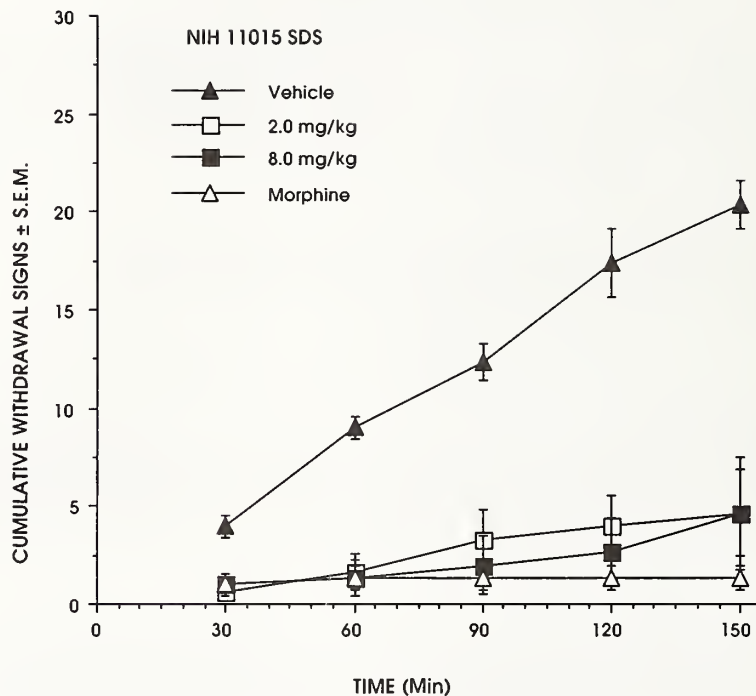
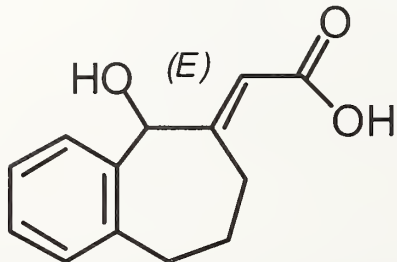


Fig NIH 11015-SDS. Results of study in which single doses of NIH 11015 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: This compound has many characteristics in common with morphine. The naloxone AD50 suggested selective mu opioid-receptor properties; however, subtype testing indicated mu-, and very weak kappa- and delta-receptor interactions.

NIH 11016 (NCS-382, GHB antagonist)



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - 0% at 1, 9.2% at 10 and 5.5% at 30
- 2) TF vs. M - 20% at 1, 5% at 10 and 0% at 30
- 3) PPQ - 2% at 1, 0% at 10 and 20 and 38% at 30
- 4) HP - 0% at 1 and 10, 12.5% at 30

NIH 11016 (Continued)

MONKEY DATA  
(SDS)

NIH 11016 produced a feeble exacerbation of withdrawal signs at the 4,16 and 32 mg doses (see Fig NIH 11016-SDS). Some jaw sag was noted at the high dose.

MONKEY DATA  
(PPt-W)

As shown in the Fig (NIH 11016 PPt-W), it is evident that NIH 11016, at 32 mg/kg s.c., did not precipitate withdrawal in morphine-dependent monkeys. Because drug supply was exhausted, n = 2.

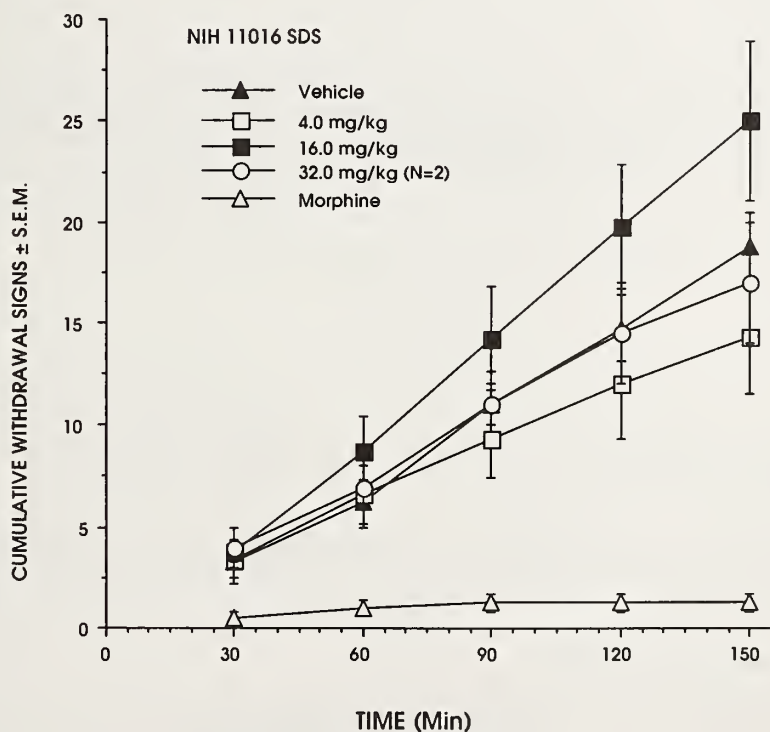


Fig NIH 11016-SDS. Results of study in which single doses of NIH 11016 were substituted for morphine in morphine-dependent monkeys in withdrawal.

NIH 11016 (Continued)

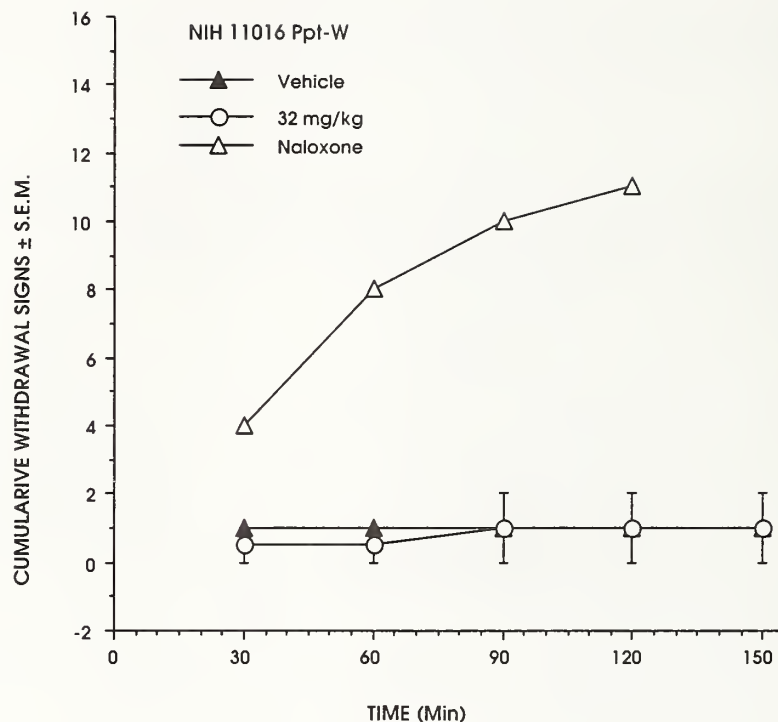
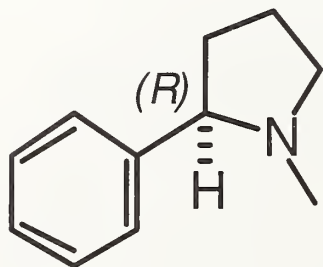


Fig NIH 11016-PPt-W. Results of study in which NIH 11016 was administered to morphine-dependent monkeys 2 hr after morphine.

Comment: Apparently, NIH 11016, a GHB antagonist, has little effect antinociceptively and neither substitutes for morphine nor acts like an opioid antagonist. Because drug supply was exhausted, no further work was done.

NIH 11017 (9801) (R)-(+)-Nicotine di-*d*-tartrate



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - 0% at 1, 28% at 10 and 0% at 30
- 2) TF vs. M - 0% at 1, 13% at 10 and 0% at 30
- 3) PPQ - 10.23 (4.53 - 23.09)
- 4) HP - 25% at 1, 10 and 30

Special Test:



NIH 11017 (Continued)

Table. Naltrindole vs ED80 of NIH 11017 in PPQ test.

Pretreatment Time		Route	% Antagonism
Naltrindole	NIH 11017		
30 min	20 min	s.c.	19% at 1, 36% at 15 and 20, 93% at 25 and 81% at 30
30 min <sup>a</sup>	20 min	s.c.	11% at 1, 28% and 9% at 10 and 11% at 30 <sup>b</sup>

<sup>a</sup>Repeated.

<sup>b</sup>On each test the mice showed tension in their front paws with toes arched. Hind feet were slightly splayed.

MONKEY DATA  
(SDS)

At low doses of 1.5 mg/kg, s.c., NIH 11017 reduced the number of withdrawal signs (see figure); the reduction was attributable to the relaxed abdominal muscles and failure to vocalize when the abdomen was palpated. However, at the high dose, 6.0 mg/kg, s.c., withdrawal seemed intensified due primarily to increased incidence of restlessness, tremors and retching. At this dose other signs were noted including salivation, jaw sag and eyelid ptosis. The drug has a dual action which may reflect multiple properties.

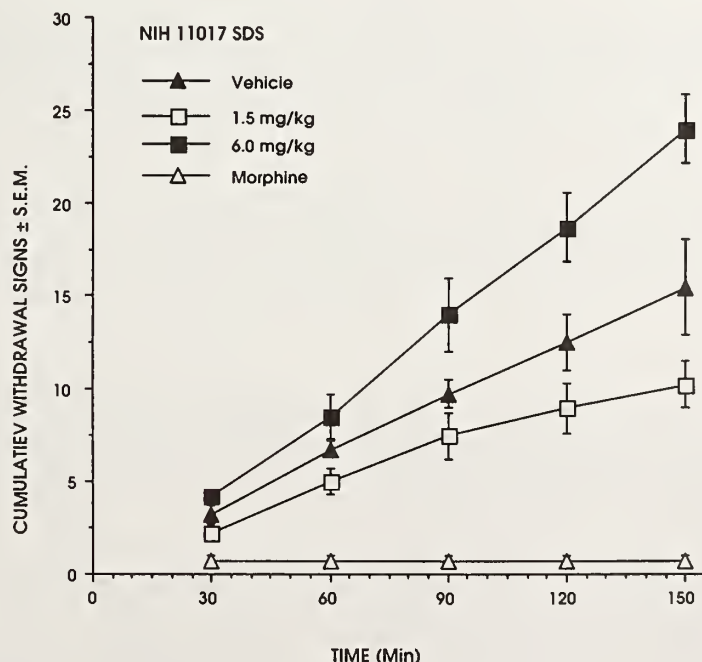
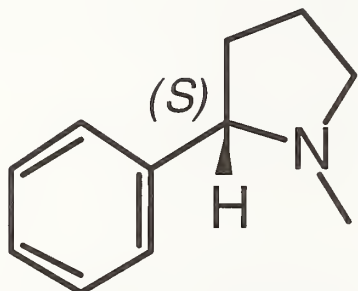


Fig NIH 11017 SDS. Results of study in which single doses of NIH 11017 were substituted for morphine in morphine-dependent monkeys in withdrawal.

**NIH 11017 (Continued)**

Comment: Although NIH 11017 showed delta-opioid receptor agonist properties in mice, the dose response with naltrindole was erratic and precluded the determination of a meaningful AD50. The side effects probably played a role. In withdrawn monkeys, a rather complex dose-dependent profile of CNS peripheral effects was observed.

**NIH 11018 (9733) (S)-(-)-Nicotine di-*l*-tartrate**



**MOUSE DATA - ED50 OR AD50**

(95 % C.L.) (mg/kg or % change)

- 1) TF – 8.91 (3.35 – 23.67)<sup>a</sup>
- 2) TF vs. M – Inactive at 1, 10 and 30<sup>b</sup>
- 3) PPQ – 1.42 (0.44 – 4.61)<sup>c</sup>
- 4) HP – 16.92 (7.05 – 40.61)<sup>d</sup>

<sup>a</sup>All mice were sedated at 10, two convulsed at 30, all were jittery.

<sup>b</sup>All mice convulsed and two died at 30, two convulsed at 10.

<sup>c</sup>One mouse died and two mice convulsed at 10, mild sedation at 3.

<sup>d</sup>One mouse died and two mice convulsed at 30, One convulsed at 20, all were sedated at 10.

Special Test:

Table. Naltrindole vs NIH 11018 (25 mg/kg) in tail-flick test.

Pretreatment Time		Route	AD50 or % Antagonism
Naltrindole	NIH 11018		
30 min	20 min	s.c.	0% at 1, 2% at 10 and 0% at 30 <sup>a,b</sup>

<sup>a</sup>One mouse died at 30.

<sup>b</sup>Naltrindole reduced the intensity of the convulsions and tremors induced by NIH 11018.

**MONKEY DATA**

(SDS)

As shown in the figure, at doses of 0.75 and 3.0 mg/kg, s.c., NIH 11018 appeared to exacerbate withdrawal signs, however, the apparent increase was due to an increased incidence of retching with both doses and at the high dose, a higher incidence of vocalization when the abdomen was palpated. In addition, the signs tremors, jaw sag and eyelid ptosis were noted.

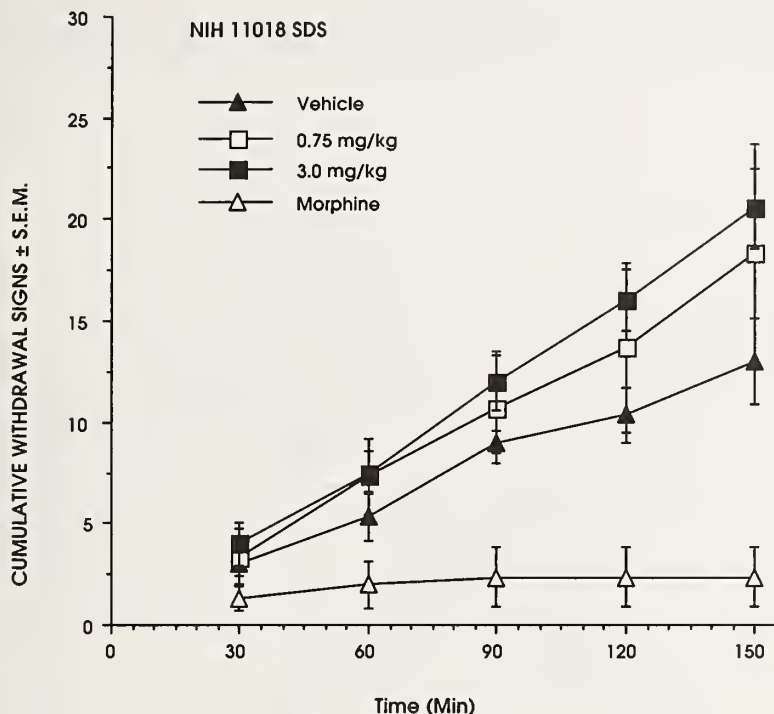
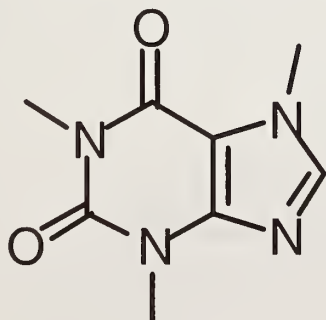


Fig NIH 11018-SDS. Results of study in which single doses of NIH 11018 were substituted for morphine in morphine-dependent monkeys in withdrawal

Comment: This compound has analgesic properties in mice that are not delta-opioid receptor related. The drug is a CNS stimulant in mice judging from the convulsions. In the monkey, NIH 11018 exacerbated withdrawal. However, the increase was associated with an increased incidence of retching and vocalization. The drug manifests many CNS stimulant and possibly peripheral effects.

NIH 11019 (10613) Caffeine tartrate



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - 0% at 1 and 10, 8% at 30
- 2) TF vs M - 0% at 1, 2% at 10 and 0% at 30
- 3) PPQ - Inactive at 1, 10 and 30
- 4) HP - Inactive at 1, 10 and 30

NIH 11019 (Continued)

MONKEY DATA  
(SDS)

As shown in the accompanying figure, NIH 11019 neither substituted for morphine nor exacerbated withdrawal at doses of 4 and 16 mg/kg.

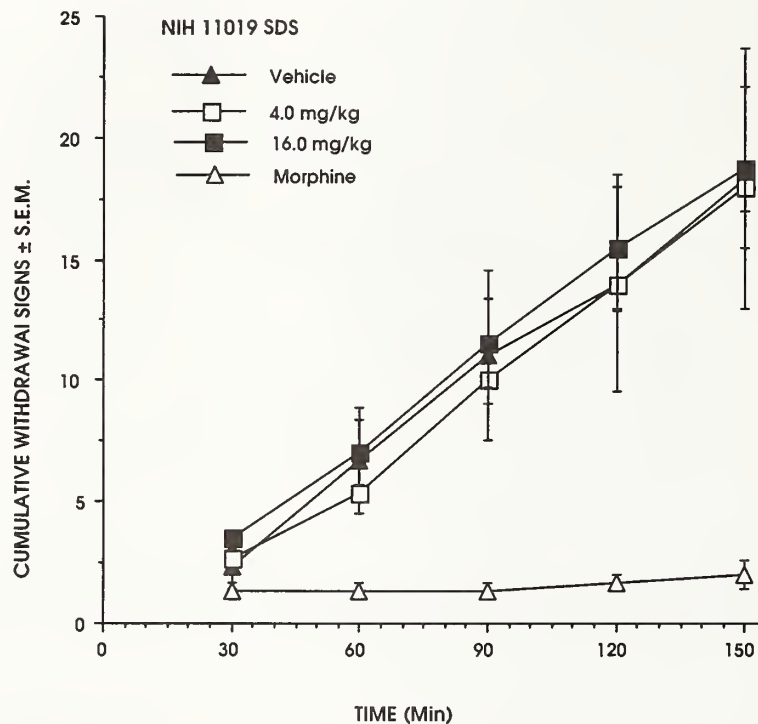
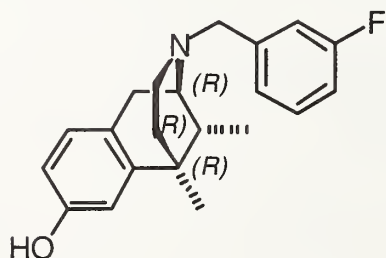


Fig NIH 11019-SDS. Results of study in which single doses of NIH 11019 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: NIH 11019 seems to be devoid of opioid properties.

NIH 11020 (-)-(1R,5R,9R)-5,9-dimethyl-2-(3-fluorobenzyl)-2'-hydroxy-6,7- benzomorphan.oxalate



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - Inactive at 1, 10 and 30
- 2) TF vs. M - 0% at 1, 12% at 10 and 3% at 30
- 3) PPQ - 26% at 1, 0% at 3, 49% at 10 and 27% at 30
- 4) HP - 13% at 1, 0% at 10 and 13% at 30

NIH 11020 (Continued)

MONKEY DATA  
(SDS)

The results illustrated in the accompanying figure indicate that at 4 and 16 mg/kg, NIH 11020 neither substituted for morphine nor exacerbated withdrawal.

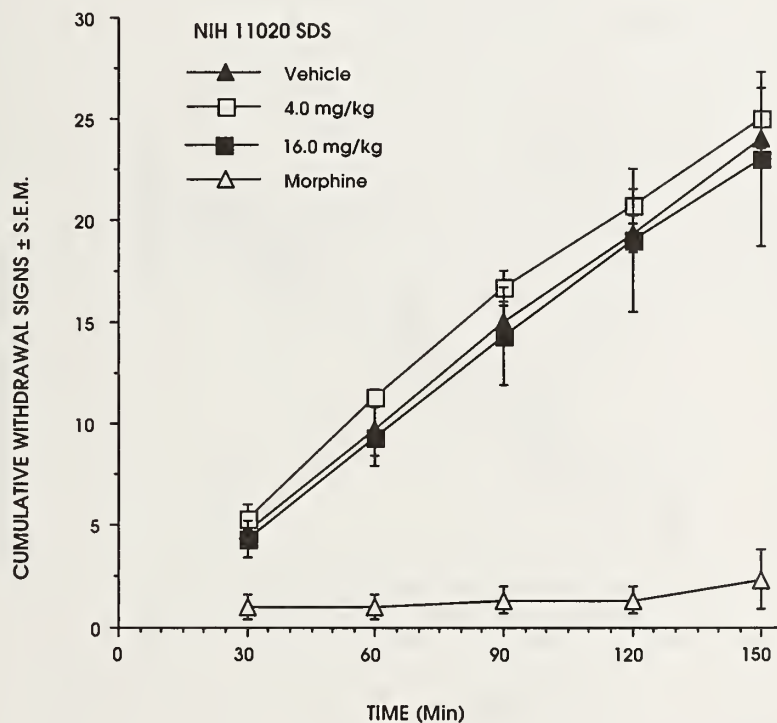
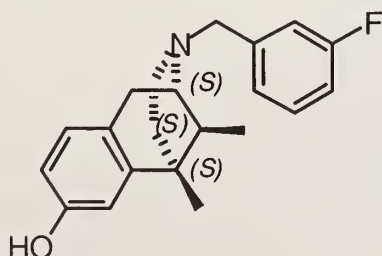


Fig NIH 11020 SDS. Results of study in which single doses of NIH 11020 were substituted for morphine in morphine-dependent monkeys in withdrawal

Comment: The results in mice and monkeys do not indicate opioid activity.

NIH 11021 (+)-(1*S*,5*S*,9*S*)-5,9-dimethyl-2-(3-fluorobenzyl)-2'-hydroxy-6,7-benzomorphan.oxalate



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - 0% at 1 and 10, 2% at 30
- 2) TF vs. M - 8% at 1, 0% at 10 and 13% at 30
- 3) PPQ - 5% at 1, 23% at 10 and 26% at 30
- 4) HP - 0% at 1, 25% at 10 and 0% at 30

NIH 11021 (continued)

MONKEY DATA

(SDS)

At 4 and 16 mg/kg, NIH 11021 neither substituted for morphine nor exacerbated withdrawal. See accompanying illustrated data.

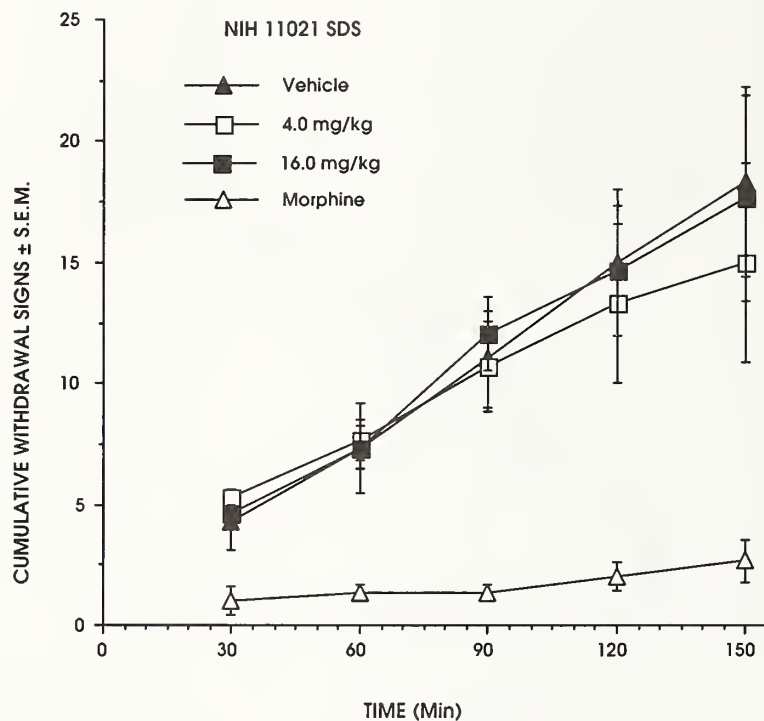
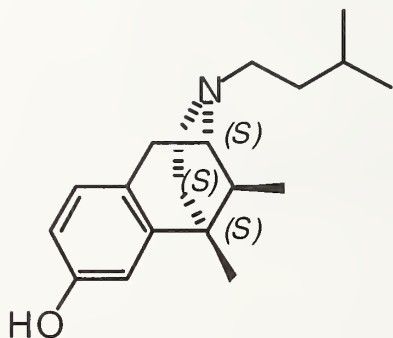


Fig NIH 11021 SDS. Results of study in which single doses of NIH 11021 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The results in mice and morphine-dependent rhesus monkey do not predict opioid properties for NIH 11021.

NIH 11022 (+)-(1*S*,5*S*,9*S*)-2-(3-Methylbutyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.oxalate



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - 1% at 1, 0% at 10 and 5% at 30<sup>a</sup>
- 2) TF vs. M - 0% at 1, 5% at 10 and 0% at 30<sup>a</sup>
- 3) PPQ - 19.36 (12.82 - 29.25<sup>a</sup>)
- 4) HP - 13% at 1, 10 and 30<sup>a</sup>

<sup>a</sup>Vehicle was 5% hydroxypropyl-β-cyclodextrin in water.

MONKEY DATA  
(SDS)

Because drug supply was exhausted, the data shown in the accompanying figure are those of one experiment. These limited data suggest that at 4 and 16 mg/kg, that NIH 11022 neither substituted for morphine nor exacerbated withdrawal.

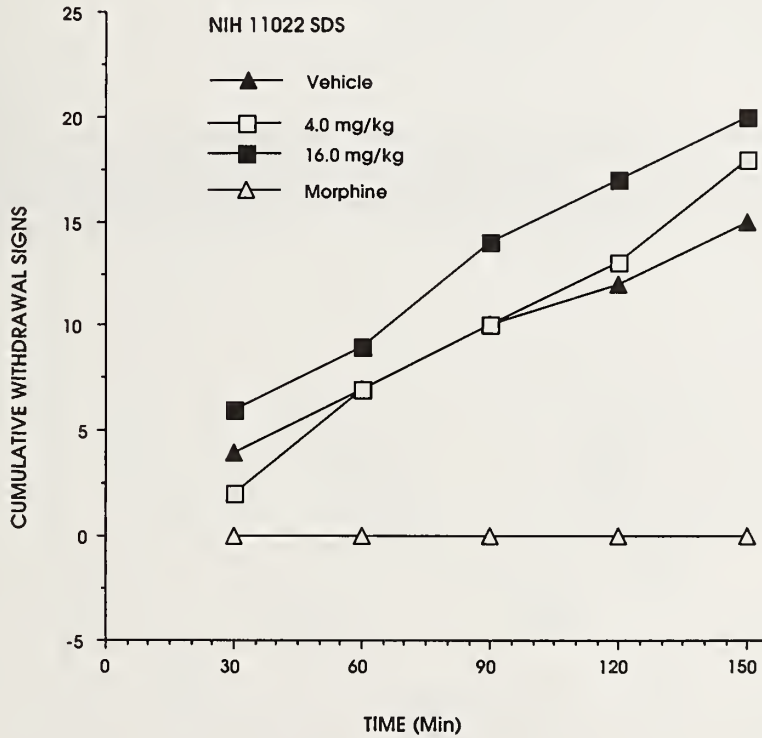
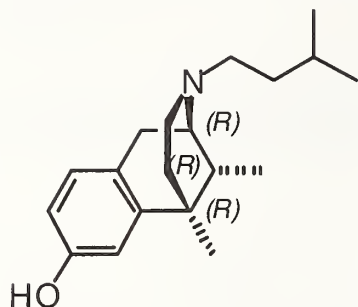


Fig NIH 11022 SDS: Results of study in which single doses of NIH 11022 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: NIH 11022 shows some weak activity in the PPQ test. The limited testing does not indicate a remarkable mu-opioid receptor interaction.

NIH 11023 (-)-(1*R*,5*R*,9*R*)-2-(3-Methylbutyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.oxalate



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - 2.5 (1.23 - 5.93)<sup>a</sup>
- 2) TF vs. M - 0% at 1 and 10, 12% at 30<sup>a,b</sup>
- 3) PPQ - 0.39 (0.23 - 0.66)<sup>a</sup>
- 4) HP - 13% at 1, 0% at 10 and 13% at 30<sup>a</sup>

<sup>a</sup>Vehicle was 5% hydroxypropyl- $\beta$ -cyclodextrin.

MONKEY DATA  
(SDS)

At doses of 2 and 8 mg/kg, NIH 11023 reduced the overall number of withdrawal signs but it did not substitute for morphine. At the high dose jaw sag, salivation and eyelid ptosis were observed. In addition one of the monkeys in the group convulsed 2 min after receiving the drug. Pentobarbital, 30 mg/kg i.p., effectively terminated the convulsion. Vehicle was 10% hydroxypropyl- $\beta$ -cyclodextrin.

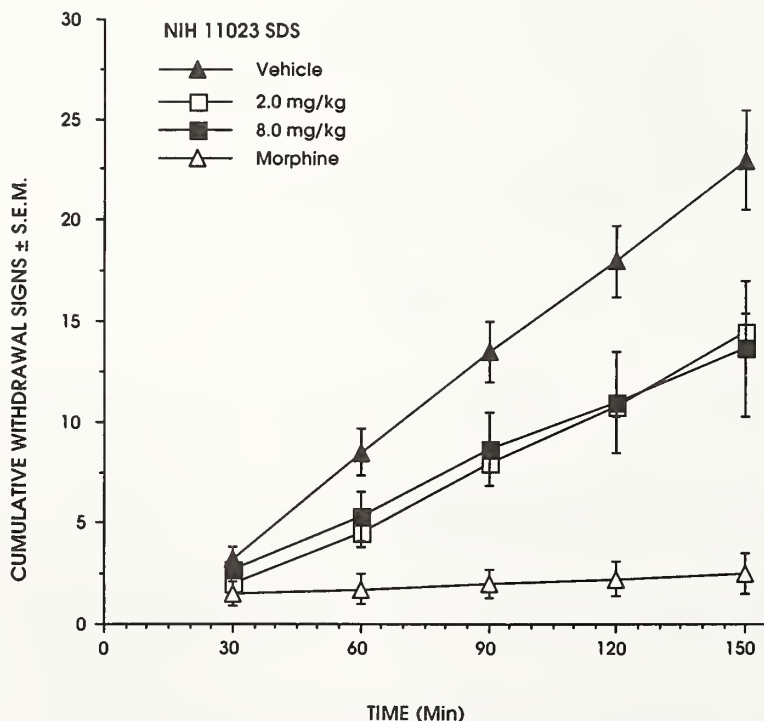
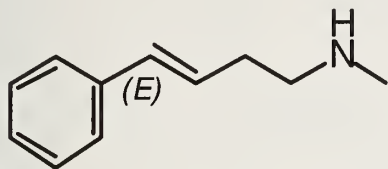


Fig NIH 11023 SDS. Results of study in which single doses of NIH 11023 were substituted for morphine in morphine-dependent monkeys in withdrawal

Comment The profile activity of this compound suggests heterogeneous opioid receptor properties (possibly kappa and delta opioid). Opioid subtype testing could resolve this issue.



NIH 11024 (10936) Metanicotine.oxalate



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - 0% at 1, 3% at 10 and 0% at 30<sup>a</sup>
- 2) TF vs. M - Inactive at 1, 10 and 30<sup>a</sup>
- 3) PPQ - 0% at 1 and 10, 26% at 30<sup>a</sup>
- 4) HP - 0% at 1, 12.5% at 10 and 30<sup>a</sup>

Intravenous (5 min pretreatment time)

- 5) TF - 0% at 1 and 10, 14% 30<sup>a</sup>

<sup>a</sup>Clonic convulsions in 6/6 at 30. 3/6 died and 3/6 recovered 5min after injection.

MONKEY DATA

(SDS)

As shown in the accompanying figure, NIH 11024 neither substituted for morphine nor exacerbated withdrawal in the dose range of 4-16 mg/kg.. Some jaw sag was observed in 1/4 monkeys at the low and high doses. Slowing was noted in 1/4 monkeys at the high dose.

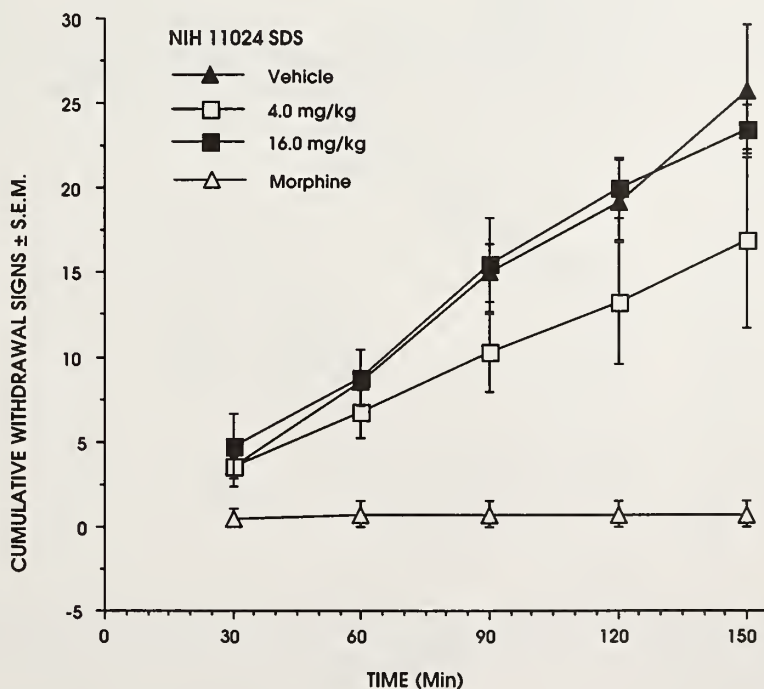
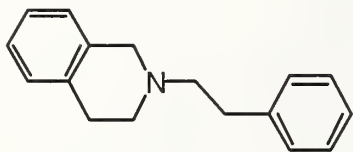


Fig NIH 11024 SDS. Results of study in which single doses of NIH 11024 were substituted for morphine in morphine-dependent monkeys in withdrawal

Comment: NIH 11024 did not display mu-opioid receptor agonist properties. In the monkey, perhaps it is a CNS depressant.

NIH 11025 2-(2-phenethyl)-1,2,3,4-tetrahydroisoquinoline.oxalate



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - 5% at 1, 14% at 10 and 26% at 30
- 2) TF vs. M - inactive at 1, 10 and 30
- 3) PPQ - 10.5 (2.1 - 54.4)
- 4) HP - 12.5% at 1, 0% at 10 and 30

MONKEY DATA  
(SDS)

At 4 and 16 mg/kg, some delayed attenuation of withdrawal signs was apparent with NIH 11025 (see accompanying figure).

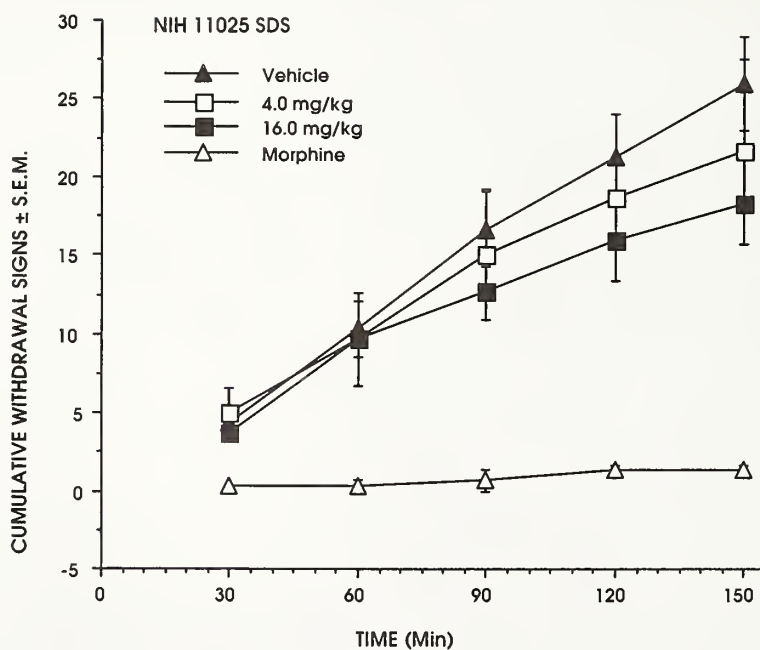
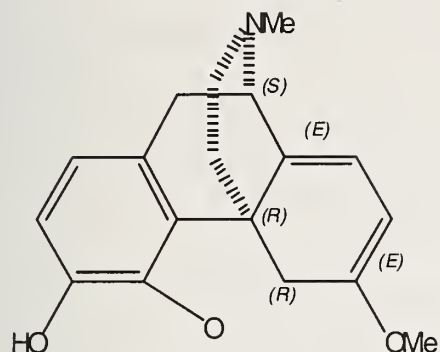


Fig NIH 11025-SDS. Results of study in which single doses of NIH 11025 were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: The results in mice and monkeys suggest some weak and/or delayed effects. Under conditions of these assays, NIH 11025 does not portend significant opioid effects.

## NIH 11026 ((+)-Oripavine)



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - 1% at 1, 20% at 10 and 29% at 30
- 2) TF vs. M - 7% at 1, 4% at 10 and 0% at 30
- 3) PPQ - 0.58 (0.14 - 2.36)
- 4) HP - 13% at 1, 0% at 10 and 38% at 30

Special Tests: Opioid subtype testing in PPQ test:

- a)  $\beta$ -FNA (i.c.v., 4 hr pretreatment) vs ED80 of NIH 11026 (s.c., 20 min pretreatment) in PPQ test: 12% at 1, 21% at 3, 29% at 10 and 46% at 30  $\mu$ g/brain.
- b) nor-BNI (s.c., 2 hr pretreatment) vs ED80 of NIH 11026 (15 mg/kg, 20 min pretreatment) in PPQ: 0% at 1, 60% at 10 and 35% at 30.
- c) Naltrindole (s.c., 30 min pretreatment) vs ED80 of NIH 11026, 20 min pretreatment) in PPQ: Inactive at 1, 10 and 30.

## MONKEY DATA

NIH 11026 partly attenuated withdrawal signs in morphine-dependent monkeys. As shown in the figure, the effect was dose-related. Onset of action was prompt; however, the duration was short

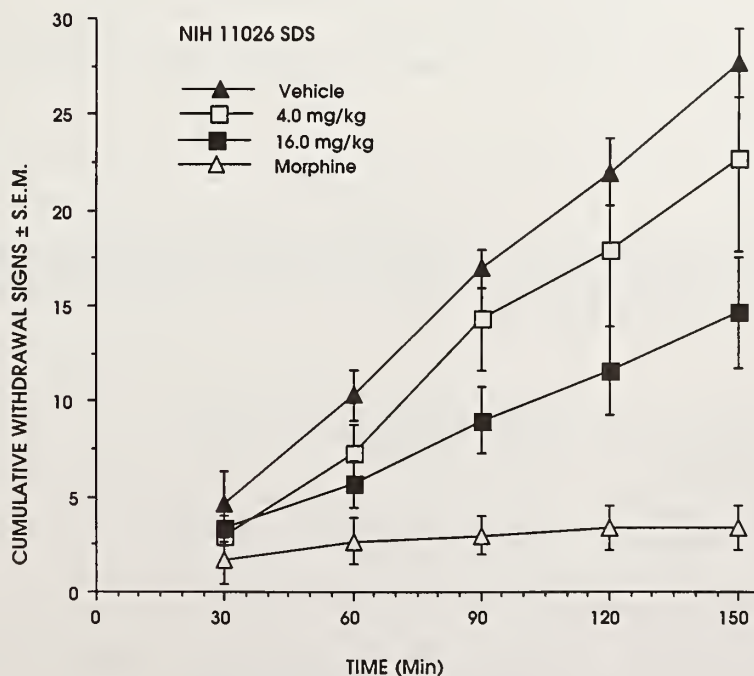
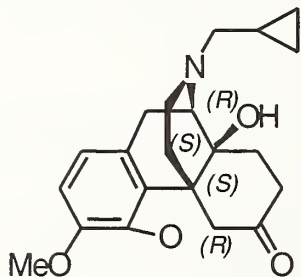


Fig NIH 11026-SDS. Results of study in which single doses of NIH 11026 were substituted for morphine in morphine-dependent monkeys in withdrawal.

**NIH 11026** (Continued)

Comment: NIH 11026 demonstrated very weak opioid activity, possibly of mu and kappa subtypes.

**NIH 11028** (3-O-Methylnaltrexone.HCl)



**MOUSE DATA - ED50 OR AD50**  
(95 % C.L.) (mg/kg or % change)

- 1) TF – 1% at 1, 0% at 3 and 3% at 30
- 2) TF vs. M – 0.47 (0.30 – 0.72)
- 3) PPQ – NT
- 4) HP – 13% at 1 and 10, 0% at 30

- Special Tests:
- 1) NIH 11028 (p.o.) vs ED80 of morphine (s.c.) in TF: AD50 = 2.31 (1.73 – 3.09)
  - 2) NIH 11028 (s.c., 6 hr pretreatment) vs ED80 of morphine (s.c.) in TF: 29% at 0.5, 9% at 1, 7% at 4 and 12% at 6.

Note: Naloxone (p.o.) AD50 vs ED80 of morphine (s.c.): 1.44 (0.51 – 4.03)

**MONKEY DATA**

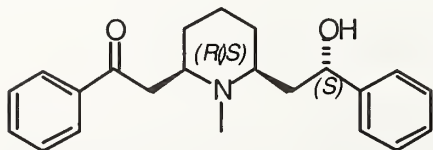
(SDS)

Not Tested.

Comment: Apparently, NIH 11028 is a non selective, orally active opioid antagonist which acts promptly. Its duration of action is less than 6 hr.

**NIH 11034** (Lobeline)

Lobeline has been found to be a short-acting antagonist at the nicotine receptor in the nucleus accumbens (Benwell, M.E. and Balfour, D.V., Br. J. Pharmacol. 125, 1115-9 (2000)). Knowing of nicotine's interaction with opioids, we decided to evaluate this compound for similar properties.



**MOUSE DATA - ED50 OR AD50**  
(95 % C.L.) (mg/kg or % change)

- 1) TF – 0% at 1, 5% at 10<sup>a</sup>
- 2) TF vs. M – Inactive at 1 and 10
- 3) PPQ – 3.01 (1.36 – 6.70)
- 4) HP – 0% at 1, 13% at 10 and 13% at 30<sup>b</sup>

<sup>a</sup>Five of six convulsed and died at 30 mg/kg. At 10 mg/kg there was decreased locomotor activity.

<sup>b</sup>Eight of eight convulsed at 30 mg/kg; four of eight died at this dose. At 10 mg/kg there was decreased locomotor activity.

MONKEY DATA  
(SDS)

At doses of 1 and 4 mg/kg s.c., lobeline had little effect regarding attenuation of withdrawal (see figure). It did relax abdominal muscles but increased the incidence of retching.

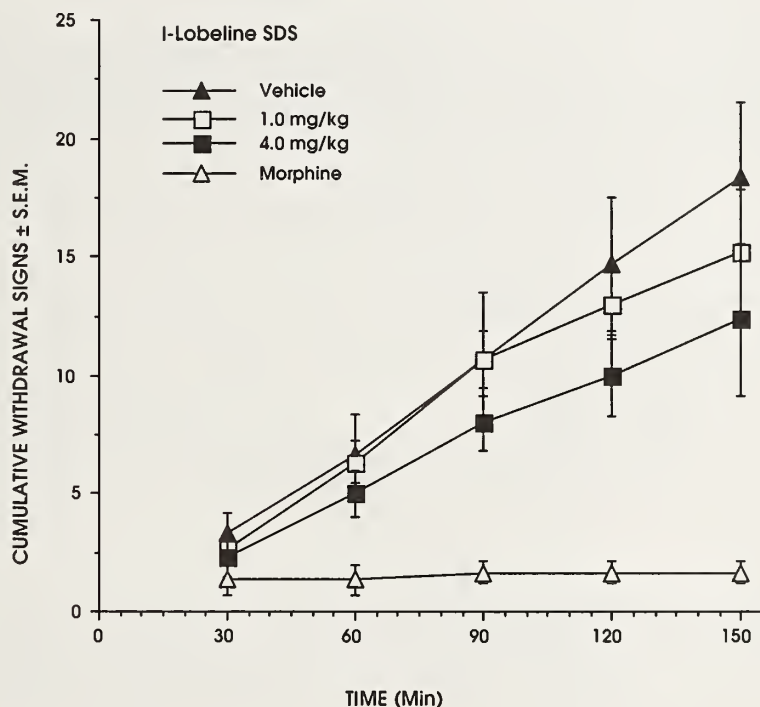
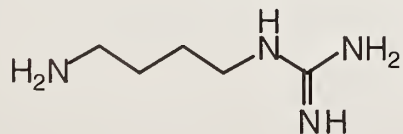


Fig I-Lobeline-SDS. Results of study in which single doses of lobeline were substituted for morphine in morphine-dependent monkeys in withdrawal.

Comment: Lobeline resembles the action of nicotine regarding convulsions in mice and PPQ activity. In the monkey it also displayed nicotine's profile with reference to muscle relaxation and retching.

NIH 11035 (Agmatine Sulfate)



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF - a) Inactive at 1, 10 and 30<sup>a</sup> (s.c.)  
b) 2% at 1, 32% at 10 and 31% at 30 (µg/brain, i.c.v.)
- 2) TF vs. M - Inactive at 1, 10 and 30<sup>a</sup>
- 3) PPQ - a) 38% at 1, 40% at 10 and 0% at 30<sup>a</sup> (s.c.)  
b) 3% at 0.1, 13% at 0.3, 16% at 1, 50% at 3, 41% at 10, and 53% at 30 µg/brain (i.c.v.)
- 4) HP - 0% at 1, 20% at 10 and 38% at 30<sup>a</sup>

## NIH 11035 (Continued)

Evidence suggests that agmatine, an endogenous metabolite of L-arginine, is an important neurotransmitter in mammals. It binds to  $\alpha_2$ -adrenoreceptor and imidazoline binding sites, blocks NMDA receptor channels and other cationic channels. It inhibits tolerance to and withdrawal from morphine and has been reported to block alcohol withdrawal in dependent rats (Reis, D.J. and Regunathan, S., Trends Pharmacol. Sci., 21,187-93 (2000) and Uzbay, I. T., Yesilyurt, O., Celik, T., Ergun, H. and Isimer, A., Behav. Brain Res., 107, 153-9 (2000).

### MONKEY DATA (SDS)

The data are illustrated in Fig NIH 11035 SDS. Kruskal-Wallis ANOVA indicated significance ( $P = 0.05$  or less) differences among the treatments at each time interval. Post hoc comparison using the Mann-Whitney U test revealed significant differences between morphine and each treatment group at each time interval. Agmatine, at the low dose of 6 mg/kg, showed a strong trend ( $p = 0.17$  to  $0.06$ ) regarding attenuation of withdrawal signs at each time interval.

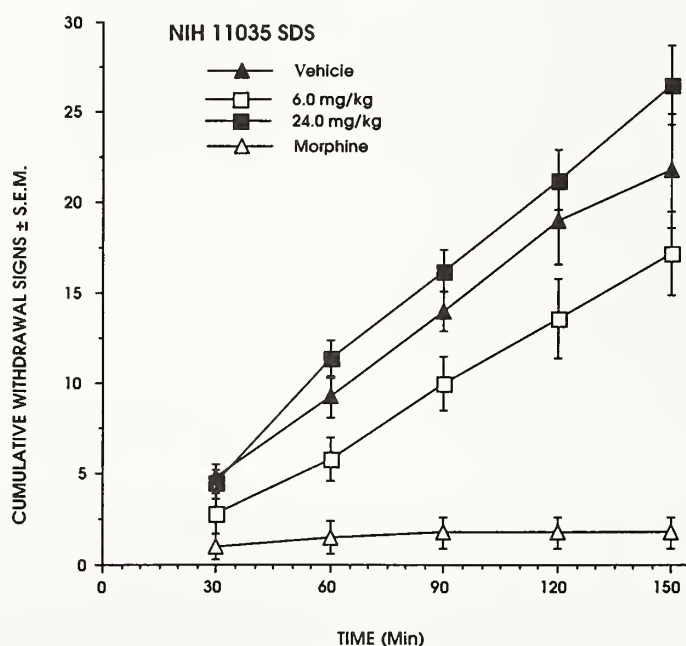
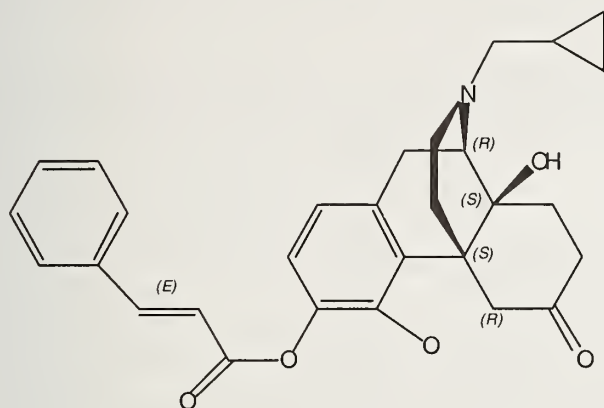


Fig NIH 11035 SDS. Results of study in which single doses of NIH 11035 were substituted for morphine in morphine-dependent monkeys in withdrawal

Comment: Agmatine may require more fine-tuning to characterize its interaction with the opioid system.

**NIH 11037 (3-O-Cinnamoylnaltrexone.HCl)**



MOUSE DATA - ED50 OR AD50  
(95 % C.L.) (mg/kg or % change)

- 1) TF – Inactive at 1, 10 and 30<sup>a</sup>
- 2) TF vs. M – 0.013 (0.003 - 0.04) 30 min
- 3) PPQ – Inactive at 30<sup>a</sup>
- 4) HP – 13% at 30<sup>a</sup>

Special 4-hr pretreatment study (s.c.) - Naltrexone and NIH 11037 vs morphine.

Naltrexone	NIH 11037
AD50 = 1.92 (0.69 - 5.31)	2.69 (0.99 - 7.30)

Subtype testing as a kappa antagonist:

NIH 11037 AD50 vs ED80 of enadoline, a kappa agonist = 0.196 (0.045-0.849).

MONKEY DATA  
(PPT-W)

NIH 11037 precipitated withdrawal in morphine-dependent monkeys at doses of 0.03 and 0.15 mg/kg. As shown in the accompanying figure, this drug appeared to be more potent than naloxone, the reference standard. Onset of action was rapid and offset seemed longer than that of naloxone.

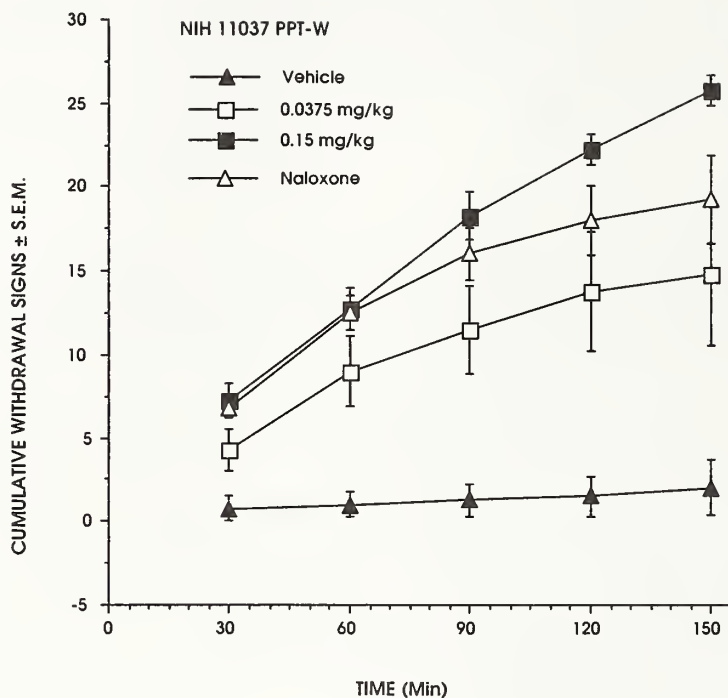


Fig. NIH 11037 Results of study in which NIH 11037 was administered to morphine-dependent monkeys (PPT-W).

Comment: Based on the results of studies in mice and morphine-dependent monkeys, we conclude that NIH 11037 is a potent mu- and kappa-opioid receptor antagonist. Whether or not this drug also has delta-opioid receptor antagonist activity remains to be determined.

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