INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

STUDIES ON THE CHANGES OF NEUROTRANSMITTERS AND NEUROPEPTIDES IN THE BASAL GANGLIA OF THE RAT BRAIN INDUCED BY THE PSYCHOSTIMULANTS METHAMPHETAMINE AND COCAINE

by

YONG ZHANG

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1999

This manuscript has been read and accepted for the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

12/14/98
Date

Chair of Examining Committee
Dr. Jesus Angulo, Hunter College

12/16/98
Date

Executive Officer
Dr. Richard L. Chappell

Dr. Thomas Schmidt-Glenewinkel, Hunter College

Dr. Ann Henderson, Hunter College

Ilya Glezer, City University of New York Medical School

Dr. Bruce McEwen, Rockefeller University

Supervising Committee

The City University of New York

ABSTRACT

STUDIES ON THE CHANGES OF NEUROTRANSMITTERS AND NEUROPEPTIDES IN THE BASAL GANGLIA OF THE RAT BRAIN INDUCED BY THE PSYCHOMOTORSTIMULANTS METHAMPHETAMINE AND COCAINE

by

Yong Zhang

Advisor: Professor Jesus A. Angulo

Methamphetamine (METH) and cocaine are the most commonly abused psychomotorstimulants. Repeated injections of psychostimulants produce behavioral sensitization. Tyrosine hydroxylase (TH) mRNA levels in the somatodendritic regions and preprotachykinin (PPT) and preproenkephalin (PPE) mRNA levels in the dopaminergic terminal region were measured to evaluate the involvement of striatal neuropeptides in behavioral sensitization. *In vivo* microdialysis was used to determine if the augmented behavioral response following daily psychostimulant administration was associated with an increase in dopamine and glutamate release in the caudate-putamen (dCPu), nucleus accumbens (NAc), ventral tagmental area (VTA) and substantia nigra compacta (SNc).

TH mRNA level in the VTA was increased at day 15 withdrawal following 6 daily (twice/day) injections of METH (4 mg/kg). The increase in the TH mRNA level in the VTA was prevented by concurrent pretreatment with the NMDA receptor antagonist MK-801 (0.1mg/kg), suggesting the involvement of glutamatergic transmission in METH-induced alterations of tyrosine hydroxylase mRNA levels.

Progressive treatment with METH (4 mg/kg) elevated PPT mRNA levels in the dCPu and the NAc between days 1 and 6 of treatment; however, PPT mRNA levels returned to control values 15 days after cessation of treatment. Coadministration of the NMDA receptor antagonist MK-801 attenuated the elevation of PPT mRNA observed after chronic treatment with METH for 6 consecutive days. In contrast, PPE mRNA levels

were significantly affected by acute treatment of METH and decayed by day 6. It is likely that the tachykinin peptides may not subserve a neuroadaptive role sustaining enduring sensitization to amphetamine, but play a role in the progressive augmentation of locomotor activity elicited by this class of drug.

Acute injection of METH (1 mg/kg) and cocaine (10 mg/kg) resulted in an elevation of dopamine efflux in all the regions measured. A METH challenge injection after 4 days withdrawal following 7 daily injections produced an even greater increase in dopamine release in the dCPu and long-lasting dopamine release in the NAc. Whereas, a cocaine challenge caused decreases and no significant changes in dopamine release in the dCPu and the NAc, respectively. In the somatodendritic regions, METH and cocaine challenges tend to decrease dopamine release compared with those induced by acute injections. Our results suggest that behavioral sensitization can be sustained during early withdrawal in the absence of augmentation in dopamine release in response to psychostimulant challenge.

Acute injection of METH (1 mg/kg) and cocaine (10 mg/kg) did not change glutamate efflux significantly in the neostriatum. Cocaine challenge increased glutamate release in both the dCPu and the NAc. In contrast, METH challenge increased glutamate release in the dCPu, but decreased glutamate efflux in the NAc. In the VTA and the SNc, acute METH decreased glutamate efflux whereas acute cocaine increased glutamate release. Challenge injections of METH and cocaine tend to enhance glutamate release. These data indicate that the neurotransmitter glutamate may be involved in behavioral sensitization to psychomotorstimulants.

ACKNOWLEDGMENTS

I wish to express my appreciation to my supervisory committee, Dr. Bruce McEwen of the Rockefeller University, Dr. Ilya Glezer of the City College of New York, and Dr. Ann Henderson of Hunter College for their support and advice.

Most of all, I would like to thank my mentor, Professor Jesus A. Angulo, for the scientific guidance and training he gave me. Not only does Dr. Angulo strive to stay in the forefront of science, but is also keen and generous to share his knowledge with us. Throughout my years in Hunter College, Dr. Angulo has always been there, supporting, encouraging and helping me. I feel lucky to have worked with him on the behavioral sensitization project. Without his help, I would not have finished my thesis.

I am also grateful to Professor Thomas Schmidt-Glenewinkel, a scientist who is knowledgeable, generous, and truly enjoys science, for his advice and help. I admire his dedication and his very sense of duty to his work.

I would also like to thank my fellow student Madelyne, Holly, Perrier, Thomas, Rudy, Pabel, Sonia. My study would not be so smooth without their help. They are the best labmates I can found in the world and I enjoy working and having fun with them.

Special thank to my parents and my sister, for their understanding and support throughout these years.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

METH	Methamphetamine
TH	Tyrosine Hydroxylase
CCK	Cholecystokinin
PPCCK	Preprocholecystokinin
PPT	Preprotachykinin
SP	Substance P
PPE	Preproenkephalin
ENK	Enkephalin
dCPu	Caudate-putamen
NAc	Nucleus Accumbens
VTA	Ventral Tagmental Area
SNc	Substantia Nigra Compacta
SNr	Substantia Nigra Reticulata
GP	Globus Pallidus
STN	Subthalamic Nucleus

CHAPTER I

Introduction

Abuse History of Psychomotorstimulant

1. Abuse history of amphetamine

Man's use of amphetamine-like compounds for medicinal purposes has a long history that dates back to ancient times. In China, more than 5000 years ago, traditional medical doctors treated nasal congestion with an herbal tea brewed from the ephedra plant. The plant's decongestant properties, derived from the stimulant ephedrine, were developed in a synthetic form as alpha methyl phenyl ethylamine (amphetamine) in 1887. Amphetamine sulfate was later introduced into the market, as a decongestant, in the form of a Benzedrine inhaler in 1932.

Its subsequent easy availability produced a wide spread abuse of amphetamine. For example, people chewed and swallowed the amphetamine containing cotton plugs in the Benzedrine inhalers, and in1935, the development of tablets to treat narcolepsy resulted in an even greater drug abuse. In the 1940's, not only had amphetamine been widely prescribed in the medical profession, it was also used by the American military to forestall sleep, and maintain a heightened level of alertness. Student in the 50's and 60's, used amphetamine to remain awake on preexam nights, and truck drivers dosed themselves with amphetamine to enhance their alertness on long distance trips (Rawlins, 1968). By the early 1970's, amphetamine use in the United States had peaked; 10 billion amphetamine tablets were legally produced (Grinspoon and Hedblom, 1975). After that, amphetamines were gradually replaced by cocaine, however, in recent years, there has been a surge in the use of amphetamines again.

2. Abuse history of cocaine

Cocaine is an alkaloid found in the leaves of the shrub *Erythrozylon coca* and *E. novogranatense*. Similar to amphetamine use, the ancestors of the Incas started to chew coca leaves at least 5,000 years ago (Johanson and Fischman, 1989; Van Dyke and Byck, 1982). In 1855, cocaine was first isolated by the German chemist Friedrich Gaedcke, and then characterized by Albert Niemann in 1859. Since then, cocaine has become popular in western society, and had been recommended for the treatment of alcoholism, morphine addiction and depression (Rosecran and Spitz, 1987). Cocaine was widely used in the United States in the late nineteenth century and early twentieth century (Das and Laddu, 1993), and its abuse started to appear in the country when cocaine containing products such as cigarettes, and beverages like "Coca Cola" were introduced into the market. In 1914, Congress passed a law prohibiting the inclusion of cocaine in medicine, and restricted the importation and sale of cocaine. However, it continued to be used particularly among artists and musicians from the 20's to the 60's (Petersen, 1977). Since the 70's, cocaine abuse has increased in the United States. Based on a 1990 survey conducted by the National Institute on Drug Abuse, about 1.6 million people were current cocaine users; 6 million had used cocaine in the previous year, and nearly 23 million people used cocaine at least once in their lifetime (National Institute on Drug Abuse, 1991).

Studies on Reward Center of the Brain

1. Brain stimulation studies

After the sensory and motor systems had been mapped in the brain by physiologists, there was doubt about whether higher centers controlling emotional feelings existed in the brain. Researchers found that it was difficult to measure an animal's higher feelings. In general, it was believed that the basic motivations-pleasure, pain and fear required the excitation of the whole brain (Olds, 1956). In the early 50's, with the help of a brain probing technique (A thin needle-like electrode is inserted into a particular brain region without damaging the superficial structures, and the animal is then studied under normal circumstances) developed by Hess and a motivation measuring technique created by Skinner (A technique used to measure the rewarding effect of a stimulus according to frequency), James Olds and colleagues conducted a series of experiments to explore the systems in the brain whose functions were unknown at that time. They attempted to locate areas in the brain where stimulation is favored or avoided by animals.

Their studies found that electrical simulation of the rhinencephalon could direct animals to any spot of the experimental environment at the will of the experimenters. In subsequent experiments, animals were trained to self-stimulate electric current to specific brain regions. It was shown that animals self-stimulated at a rate of 200 to 5000 times an hour when the electrode was implanted in most parts of the mid-line system. However, response rates were around the chance level of 10 to 25 times an hour when electrodes were placed in the motor and sensory regions of the brain. Moreover, electric stimulation in some parts of the brain seemed to be more rewarding than food. The findings of these studies suggested that the brains have reward centers where emotional and motivational mechanisms are located.

2. Drug alterations of brain stimulation reward potency

After the influential discovery of the brain reward centers by Olds' group, further investigations suggested that in addition to the midline system, a multisynaptic system and subsystem may be involved in the reward function (Olds & Olds, 1965; Heath, 1963, 1972). Indeed, researchers found that stimulation of diencephalic, telencephalic circuitry, a variety of brain stem sites, and parts of the cerebellum were rewarding (Olds & Olds, 1963; Simon et al., 1975; Corbett et al., 1982). Among the various reward sites, stimulation of the sites along the medial forebrain bundle, and the ventral tegmental area produced the most robust rewarding effect (Olds & Olds, 1963). Stimulation of the medial forebrain bundle may activate the mesocorticolimbic dopamine system due to the following reasons: 1) Axons of dopamine neurons originating from the ventral tagmental area (VTA) ascend to the neostriatum via the medial forebrain bundle (Ungerstedt, 1971; Corbett & Wise, 1980; Wise, 1981). 2) Blockade of the dopamine system prevents the rewarding effect of brain stimulation (Fouriezoa & Wise, 1976). 3) Terminals of the medial forebrain bundle may form synapses with dopamine cells in the VTA and substantia nigra compacta (Wise, 1980).

3. The reward center of the brain

It was hypothesized that the substrates of rewarding brain stimulation may play an important role in both natural rewards such as food and sex as well as in drugs of abuse. The site of brain stimulation may represent substrates for drugs of abuse because addictive substances enhance responding for electrical stimulation of

brain reward sites by lowering threshold for rewarding responses (Esposito et al., 1980; Bain & Kornetsky, 1987; Kornetsky & Esposito, 1979; Hubner & Kornetsky, 1992; Bain & Kornetsky, 1989). Activation of medial forebrain bundle, which has the strongest stimulating rewarding effect, stimulates mesocorticolimbic dopaminergic systems (Wise, 1980), while drugs that alter the potency of brain stimulation reward are dopamine agonists or antagonists (Wise & Rompre, 1989). Dopamine antagonists cause rightward shifts on the rate-frequency functional curve, indicating dopamine antagonists block the rewarding effect of electrical stimulation by increasing the rewarding thresholds of electrical stimulation (Wise, 1980). Opposite to the dopamine antagonists, addictive drugs shift the rate-frequency or rate-intensity functional curve to the left, indicating a synergistic effect with brain stimulation reward. These drugs include the psychomotorstimulants amphetamine, cocaine, morphine, and nicotine. Data from studies on the changes of brain stimulation reward thresholds showed that brain stimulation reward thresholds were decreased by the psychomotorstimulants amphetamine and cocaine, heroin, morphine and ethanol (Esposito et al., 1980; Bain & Kornetsky, 1987; Kornetsky & Esposito, 1979; Hubner & Kornetsky, 1992; Bain & Kornetsky, 1989).

The site of the rewarding effect of psychomotorstimulants appears to exist in the dopamine system too. Amphetamine is a substrate of the dopamine transporter. Once bound to the dopamine transporter molecule, amphetamine is taken up and released into the cytoplasmic compartment. The transporter site then binds to dopamine molecule in the cytoplasm, and dopamine is reversibly transported and released into the synaptic cleft. The exchange diffusion between

dopamine and amphetamine is considered to be the primary mechanism by which amphetamine releases dopamine. In addiction, amphetamine evokes release of dopamine from the vesicles by reducing the pH gradient across the vesicular membrane, thereby increasing the concentration of dopamine in the cytoplasmic pool, followed by reverse transportation of dopamine across the cell membrane (Sulzer and Rayport, 1990; Sulzer et al., 1993). Similarly, cocaine has high affinity for dopamine, NE and 5-HT transporter molecules. Cocaine interacts with these three transporter molecules, therefore inhibiting the uptake of these monoamines and increasing extracellular concentration of the monoamines (Nicokysen and Justice, 1988; Taylor and Ho, 1978). Several other abused drugs, such as morphine and nicotine also synergy with brain stimulation reward by activating the dopamine system (Devine et al., 1993, Clarke et al., 1985, Welzl et al., 1989). The most addictive drugs including psychomotorstimulants amphetamine and cocaine, opiates, and nicotine activate mesocorticolimbic dopamine system and produce synergistic effects on brain stimulation reward. Thus, the mesocorticolimbic dopamine system is most likely the substrate underlying the rewarding effects of brain stimulation as well as various addictive drugs.

4. Lesion and intracranial self-administration studies

Drugs that are addictive to humans are generally addictive to animals. Thus, experimental animals can be trained to self-administer drugs. If animals maintain the behavior to self-administer a drug, it indicates that the tested drug has the reinforcing property. Combination of self-administration and lesions in the

brain can be used to examine whether or not a specific brain region is involved in reinforcement and in maintaining self-administration behavior. Lesion studies in the CNS also support the idea that the mesocorticolimbic dopaminergic system is a critical substrate for the reinforcing effects of psychomotorstimulants. Destruction of either ventral tagmental area, where dopamine cell bodies are located, or the nucleus accumbens, where dopaminergic terminals innervate, attenuate intravenous self-administration of cocaine in rats (Roberts et al., 1980, 1982; Hubner et al., 1990). Moreover, Intracranial self-administration studies showed that animals self-administrated cocaine into the medial frontal cortex, which also receives dopaminergic innervation from the ventral tagmental area, indicating that this brain region is important for the reinforcing effects of cocaine (Goeders et al., 1983, 1986).

5. Non invasive studies

In recent years, studies applying non-invasive methods such as magnetic resonance image (MRI) and quantitative [14 C] 2-deoxyglucose (2 DG) methods to characterize the neurochemical substrates of cocaine administration have been done. One of the advantages of the non-invasive method is that manipulations can be made on conscious human subjects and animals. These methods provide an opportunity to evaluate behavior simultaneously. MRI is based on the fact that certain atomic nuclei emit radio frequency signal when placed in strong magnetic fields. The signal frequencies are changed under different chemical and physical environments. MRI studies in human subjects showed that infusion of cocaine

increased the focal signals in the nucleus accumbens, caudate, putamen and ventral tagmentum while producing signal decreases in the medial frontal cortex (Breiter et al., 1997).

The 2 DG method is based on the principle that glucose is transported into neurons in the CNS, and glucose utilization is proportional to the metabolic rate of the neurons, as a result, the extent of neuronal activity can be determined. The 2DG method has been used to measure the metabolic changes under cocaine treatments. An acute and low dose of cocaine injection (0.5 -1.0 mg/kg) increases glucose utilization in the medial prefrontal cortex, nucleus accumbens and substantia nigra pars reticulata. Higher doses of cocaine (5.0 mg/kg) produce elevation in the rates of cerebral glucose metabolism in the whole brain. In contrast, chronic cocaine self-administration resulted in a decrease in the rate of metabolism in the anterior cingular cortex, which also receives dopaminergic input from the VTA. However, several components of the mesocorticolimbic system including the nucleus accumbens and the medial frontal cortex did not show significant changes in glucose utilization (Graham and Porrino, 1993). This data suggest that difference in the treatment doses and paradigms of psychomotorstimulants may result in different neurochemical alterations in the same brain area.

In summary, electrophysiological, pharmacological, lesion, intracranial self-administration, MRI and 2-DG metabolic rate studies all point to the same site in the brain- the mesocorticolimbic system, which is the neurochemical substrate involved in the addictive property of psychomotorstimulants and other abused substances such as morphine, heroin and ethanol. These findings are the main

reason that investigations on psychomotorstimulants and other abused substances have concentrated on the mesocorticolimbic dopaminergic system. It also explains why neurotransmitter dopamine is measured in our study in which the sensitized response induced by psychomotorstimulant is examined.

Behavioral Sensitization to Methamphetamine and Cocaine

1. Psychomotorstimulants induce psychosis

Humans have abused Psychomotorstimulants for over 5,000 years. Use of psychomotorstimulants can induce psychosis in those who use them. Investigators have noted marked changes in emotional states in the users of these drugs, such as depression, lethargy and high levels of anxiety, depending on differences in the dose level and time since administration (Ellinwood, 1972). Another common psychotic symptom induced by psychomotorstimulants is hallucinations including visual, auditory, olfactory or tactile (Connell, 1958; Ellinwood, 1972). Paranoia is also a dramatic symptom in psychomotorstimulant psychosis. Most individuals in paranoid state reported that someone is watching them and plotting to harm them (Griffith et al., 1970; Jonsson and Gunne, 1970). Finally, motor excitation and hyperactivity are seen in most individuals with psychomotorstimulant psychosis. Behavior induced by psychomotorstimulants includes repetitive pacing or aimless walking or driving (Gunne, 1977). The psychotic symptoms dissipate after withdrawal from the psychomotorstimulants. However, former drug users remain hypersensitive to the psychomotorstimulant even years after abstinence, since a

single dose of the same drug can precipitate psychosis easily (Robinson and Becker, 1986).

2. Animal models for psychosis

These clinical observations generated considerable interest for the development of animal models of psychomotorstimulant psychosis. Rodents are the species that were studied most thoroughly and systematically with respect to the behavioral effect of psychomotorstimulants and related neurochemical mechanism (Segal and Janowsky, 1978). Chronic exposure of moderate to high doses of psychomotorstimulants in rodents seems to mimic the stimulant abuse in human addicts (Robinson and Becker, 1986). Repeated administration of psychomotorstimulants in non-human animals induces alterations in their behaviors. The behavior produced by psychomotorstimulants depends on several factors, such as the sex of the subject, the dose and treatment paradigm. Injection of an acute dose of psychostimulants results in an increase in locomotor activity including forward locomotion, head movement, sniffing. High doses of psychomotorstimulants produce changes in locomotor activities mentioned above followed by stereotyped behavior. When a constant dose of psychomotorstimulants is injected to animals repeatedly, the behavior described above are progressively augmented. For instance, a shorter time to the onset of stereotyped behavior and more intense stereotyped behavior. This increase in behavior after repeated administration of psychomotorstimulant is termed behavioral sensitization (Robinson and Berker, 1986).

3. Behavioral sensitization to psychostimulants

Methamphetamine (METH) and cocaine are psychomotor stimulants that are among the most widely abused chemical compounds. Repeated use of METH or cocaine produces behavioral sensitization. Behavioral sensitization represents a progressive and enduring increase in locomotor activity with repeated and intermittent psychomotorstimulant treatment. Behavioral sensitization to METH and cocaine persists months after withdrawal of METH and in some cases may be permanent (Robinson and Becker, 1986). It was proposed that the sensitized response to METH and cocaine resulted from neurochemical changes that make addicts hypersensitive to psychomotorstimulants.

Much of the research on behavioral sensitization has focused on mesencephalic dopamine systems, since converging evidence suggests that mesencephalic dopamine systems play a critical role in stimulant responses to METH and cocaine. 1) Addictive drugs like methamphetamine and cocaine are dopamine releasing agents. Rats sensitized to METH and cocaine display enhanced dopamine overflow from dopamine nerve terminal field areas in the striatum, accumbens and prefrontal cortex (Robinson and Becker, 1986; Robinson et al., 1988; Kazahaya et al., 1989; Akimoto et al., 1990; Kalivas and Stewart, 1991; Patrick et al., 1991; Paulson et al., 1995). 2) Stimulation of the mesencephalic dopamine systems is necessary to induce sensitization. For example, sensitization to amphetamine and cocaine is blocked by concurrent treatment with dopamine

antagonists (Stewart and Vezina, 1989). Lesions of dopamine systems prevent sensitization to amphetamine and cocaine (Crease and Iversen, 1975; Roberts et al., 1980). 3) Injection of amphetamine directly into the ventral tegmental area, where dopamine cell bodies of mesoaccumbens and mesocorticolimbic pathways are located, induces sensitization (Kalivas and Weber, 1988; Vezina and Stewart, 1990). 4) Injection of amphetamine into the nucleus accumbens causes hyperactivity in animals previously treated with amphetamine (Hooks et al., 1992). Fifth, behavioral sensitization to amphetamine and cocaine is accompanied by hypersensitivity of dopamine D1 receptors in the nucleus accumbens as well as hyposensitivity of dendritic and somatic dopamine D2 autoreceptors in the mesencephalon (Wolf et al., 1994). Taken together, all these data suggest that mesencephalic dopamine neurons are involved in psychostimulant-induced behavioral sensitization.

In addition to alternations in the dopamine system, changes in other neuronal systems in the CNS of sensitized animals are also observed. Serotonin transmission is affected by cocaine, because cocaine inhibits presynaptic serotonin uptake (Ritz et al., 1990). The following observations argue that changes in serotonin transmission may have a role in the expression of behavioral sensitization. 1) Repeated cocaine administration increases the autoreceptor inhibition of serotonin neurons in the dorsal raphe (Cunningham et al., 1992a,b). 2) Microdialysis studies show that release of serotonin is increased during the early withdrawal period in response to cocaine challenge following repeated cocaine

treatment (Parsons and Justice, 1993). 3) Stimulation of serotonin transmission in either the VTA or NAc evokes dopamine release in the NAc (Chen et al., 1991).

Chronic administration of cocaine decreases the levels of 3 major neurofilament proteins in the VTA (Beitner-Johnson et al., 1992). The neurofilament proteins are components of the cytoskeleton and are involved in axonal transport. Reduced amount of the neurofilament proteins may lead to a decrease in the transportation of tyrosine hydroxylase from the VTA to the dopaminergic terminals in the NAc, which eventually results in a reduction in dopamine synthesis and release in the NAc.

GABAergic neurons in the nucleus accumbens and the ventral pallidum project to the VTA. Stimulation of GABA receptors in the VTA by baclofen blocked behavioral sensitization to systemic administration of cocaine, suggesting that GABAergic projections to the VTA may play a role in the development of behavioral sensitization to psychostimulant (Kalivas and Stewart, 1991). Moreover, the sensitivity of glutamate receptors in both the VTA and the NAc is changed in animals receiving repeated cocaine administration. Repeated cocaine administration leads to supersensitivity of glutamate receptors in the VTA dopamine neurons, whereas neurons in the NAc show a decrease in sensitivity to glutamate (White et al., 1993). All of these findings indicate that multiple neuronal systems in the CNS are involved in behavioral sensitization to psychomotorstimulants.

Mesencephalic Dopamine Systems

1. Dopamine synthesis, storage, release and metabolism

Dopamine synthesis begins with the precursor molecule tyrosine, an aromatic amino acid. The first step in dopamine synthesis is the conversion of tyrosine to L-DOPA by tyrosine hydroxylase (TH) (Nagatsu et al., 1964). L-DOPA is subsequently decarboxylated into dopamine. DOPA decarboxylation is catalyzed by the enzyme aromatic amino acid decarboxylase. Once dopamine is synthesized, it is packaged into vesicles by the transporter protein located on the vesicular membrane. This process is driven by electrochemical gradient across the vesicular membrane (Winkler et al., 1986, 1987). Stimulus-evoked dopamine release has been shown to occur through the mechanism of vesicular exocytosis (Trifaro et al., 1992). Exocytosis is a calcium dependent process. When an action potential reaches dopamine nerve terminals, calcium from the extracellular space moves into the nerve terminal. The influx of calcium results in the fusion of the dopamine containing vesicles with the terminal membrane, thereby releasing dopamine into the synaptic cleft (Arbuthnott et al., 1990).

After dopamine is released into the synaptic cleft, it acts on dopamine receptor, and is simultaneously taken up by dopamine transporter molecule back into the presynaptic terminal (Horn, 1990). The dopamine transporters are located on the terminal membrane and carry dopamine in either direction depending on the concentration gradient. Thus, dopamine transporters play an important role in terminating the action of dopamine as well as recycling dopamine into the nerve terminals. Additionally, catechol-o-methyltransferase (COMT) and monoamine

oxidase (MAO) in the extracellular space convert released dopamine into homovanillic acid (HVA). Also dopamine is metabolized into dihydroxyphenylacetic acid (DOPAC) by (MAO) inside the nerve terminals (Kopin, 1985). HVA and DOPAC are the primary metabolites of dopamine in the CNS, thereby being used as an index of the functional activity of the dopamine neurons in the brain.

2. Major dopaminergic pathways

In the central nervous system the majority of dopamine neurons are located in the ventral mesencephalon from which two major dopaminergic projections originate: the nigrostriatal dopamine system and the mesocorticolimbic dopamine system. The nigrostriatal pathway originates from dopaminergic neurons in the substantia nigra compacta. These dopaminergic neurons project axons that innervate the caudate-putamen of the forebrain. Some neurons also project to amygdala, septum, olfactory tubercle and cortex (Fallon and Moore, 1978). The nigrostriatal pathway has been linked to motor activity and stereotyped behaviors (for review see Angulo and McEwen, 1994).

The mesocorticolimbic pathway emanates from dopamine cell bodies of the ventral tegmental area and projects to the nucleus accumbens and prefrontal cortices. The mesocorticolimbic pathway has been implicated in locomotion, drug reinforcement and reward (Self and Nestler, 1995). Pharmocologic and behavioral studies have demonstrated that METH and cocaine administration affects both nigrostriatal and mesocorticolimbic systems (Kalivas, 1993).

3. Dopamine receptors

Five dopamine receptors have been cloned so far, but only D1 and D2 dopamine receptors have been characterized by their stimulatory and inhibitory effects on adenylyl cyclase activity (Kebabian and Calne, 1979). In the neostriatum, D1 receptors are present in the medium spiny neurons coexpressing the neuropeptides substance P, substance K and dynorphin. Whereas D2 dopamine receptors are located in the cell membrane of medium spiny projecting neurons containing peptide enkephinin (Gerfen, 1992). Lesion studies show that D2 dopamine receptors are also found in the dopamine terminals originating from the ventral mesencephalon. Eletrophysiological studies reveal that activation of dopamine receptors by stimulating nigrostriatal pathway or iontopheratic administration of dopamine into the striatum results in both inhibitory and excitatory effects on the striatal neurons (Kitai et al., 1976; Herrling and Hull, 1980).

In the ventromedial mesencephalon, D1 dopamine neurons are found in both the VTA and SN. Lesion studies show that D1 receptors are located primarily in the striatonigral terminals (Altar and Hauser, 1987; Porceddu et al., 1980). Indeed, *in situ* hybridization studies reveal that D1 mRNA is not expressed by the neurons in the ventral mesencephalon (Mansour et al., 1992). Stimulation of D1 receptors enhances neurotransmitter release from the striatonigral terminals. On the other hand, D2 dopamine receptors are found in the dopamine parikarya, and this view is further supported by the presence of D2 mRNA in dopamine neurons

in the ventral mesencephalon (Meador-Woodruff et al., 1991). Stimulation of these receptors causes fast inhibitory post-synaptic potentials (IPSP) (Lacey et al., 1987). These D2 dopamine receptors function as autoreceptors, thereby regulating dopamine synthesis, release and cell firing rate.

Basal Ganglia

1. Components of the basal ganglia

The basal ganglia are a group of nuclei located in the subcortical regions. These nuclei are highly interconnected and project to numerous brain regions as well. The components of the basal ganglia include the caudate-putamen (dCPu), nucleus accumbens (NAc), globus pallidus (GP), subthalamic nucleus (STN), entopeduncular nucleus (EPN) and substantia nigra (SN). The CPu the NAc are similar in their structures, together they are called the neostriatum and represent the input structures of the basal ganglia. The GP receives input from and lies posterior to the neostriatum. The STN is a small structure that is between the GP and SN. The SN is located in the midbrain and is composed of two areas: the substantia nigra reticulata (SNr) containing GABAergic neurons and substantia nigra compacta (SNc) containing dopaminergic neurons. The EPN and SNr make up the major output structure of the basal ganglia.

2. Neostriatum

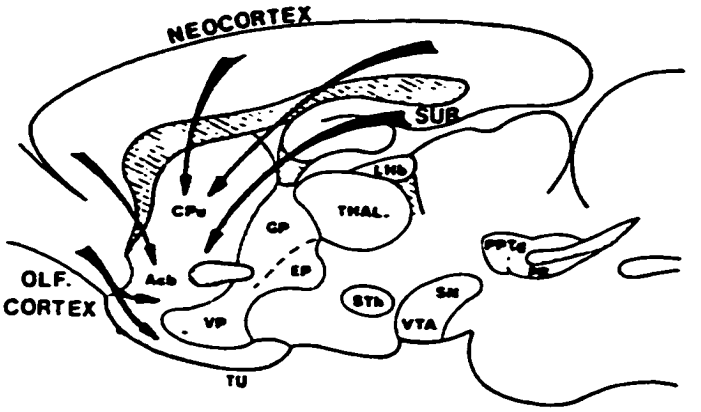
A principal component of the basal ganglia is the neostriatum, which comprises the dCPu and the NAc. The major striatal projection units are the medium spiny neurons that account for approximately 90-95% of striatal neurons (Gerfen et al., 1990). All of the medium spiny neurons utilize GABA as a neurotransmitter (Kita and Kitai, 1988). Medium spiny neurons receive massive input from the cerebral cortex and thalamus (Bouyer et al., 1984; Jayaraman, 1984). Cortical and thalamic inputs utilize glutamate as a neurotransmitter and provide excitatory input to the striatum (Kitai et al., 1976). Another major source of input to the medium spiny neurons is the dopaminergic fibers from the midbrain dopaminergic cell bodies (Fallon and Loughin, 1982; Fallon and Moore, 1978). It is believed that the responsiveness of striatal neurons to cortical and thalamic input is modulated by this dopaminergic innervation (Gerfen, 1992).

3. Effects of dopamine on the direct and indirect striatonigral pathways in the basal ganglia

The effect of dopamine on striatal neurons is mediated by at least two dopamine receptor subtypes, namely dopamine D1 and D2 receptors (Gerfen, 1992). The medium spiny neurons are separated into two major types on the basis of their axonal projections as well as by neuropeptide content and dopamine receptor expression (Gerfen and Young, 1988; Gerfen, 1992; Gerfen et al., 1990). Neurons that express tachykinin and dynorphin peptides have D1 dopamine receptors and project to both substantia nigra pars reticulata and entopeduncular nucleus; this represents the direct striatonigral pathway and provides principally

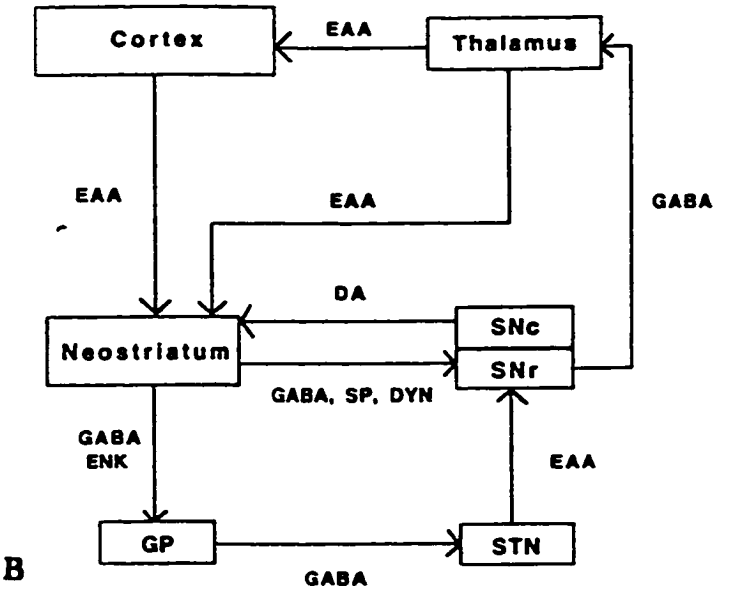
inhibitory inputs to the GABAergic neurons in the SNr and EPN, and disinhibit its target nuclei in the thalamus (Deniau and Chevalier, 1985). Neurons that express enkephalin peptides contain D2 dopamine receptors and project to the GP. GABAergic neurons in the GP in turn provide inhibitory inputs to the STN that provides an excitatory input to the substantia nigra reticulata. This represents the indirect striatonigral pathway. It is responsible for the tonic activity of GABAergic neurons in the substantia nigra reticulata and inhibition of its target nuclei in the thalamus (Robledo and Feger, 1990; Gerfen 1992; Angulo and McEwen, 1994). Thalamic neurons in turn project to the motor cortex and regulate locomotion (Deniau and Chevalier, 1985). Dopamine projections from the midbrain exert opposing effects on the direct and indirect striatonigral pathways. Dopamine seems to have an excitatory influence on striatal neurons containing GABA and tachykinin, but has an inhibitory effect on striatal neurons containing GABA and enkephalin (Gerfen et al., 1990; Li et al., 1990). Stimulating of D1 dopamine receptors increases the firing rate in the direct striatonigral neurons (Twery et al., 1991; Weick and Walters, 1988). By contrast, D2 agonist LY 171555 depresses the firing rate of indirect striatonigral neurons (Carlson et al., 1990). Thus, the overall effect of dopamine on the striatum is to facilitate movement by both facilitating the direct striatonigral pathway and suppressing the indirect striatonigral pathway (Figure. 1).

4. The involvement of the basal ganglia in locomotor disorders



A

The striatonigral and striatopallidum pathways



B

Figure 1. Schematic diagram of the basal ganglia of the rat brain. A. The sagettal section of the rat brain. B. The major afferents and efferents of the basal ganglia. GP, globus pallidus; STN, subthalamus nucleus; SNc, substantia nigra compacta; SNr, substantia nigra reticulata; Acb, nucleus accumbens; Cpu, caudate-putamen; VTA, ventral tegmental area; Thal, thalamus.

Nuclei of the basal ganglia have classically been considered as primarily motor structures, because lesions of their component structures both in human patients and in experimental animals result in prominent motor deficits (Albin et al, 1989). For example, Parkinson's disease is characterized in difficulty in the initiation of movement and slowness in the execution of movement. It is caused by the degeneration of dopamine neurons in the substantia nigra pars compacta. Dopamine content is most drastically reduced in patients with Parkinson's disease. And dopamine precursor L-DOPA is effective in the treatment of Parkinson's disease. Huntington's disease is another disease of the basal ganglia. It is due to degeneration of intrastriatal and cortical cholinergic neurons and GABAergic neurons. Its major symptoms include chorea and dementia. Finally, lesion of the subthalamic nucleus, another component of the basal ganglia, also causes the motor disorder Ballism, whose symptoms are involuntary movements (Albin et al., 1989).

Tachykinin Peptides and Receptors

1. Tachykinin peptides

Tachykinins include a family of peptides named for their ability to cause a rapid contraction of the ileum. Mammalian tachykinin peptides consist of the neuropeptides substance P (SP), neurokinin A (NKA) and neurokinin B (NKB). These peptides all share the carboxyl terminal amino acid sequence -PHE-X-GLY-LEU-MET-NH2, where X represents a hydrophobic or aromatic residue. Mammalian tachykinin peptides are derived from two genes: the preprotachykinin

A and preprotachykinin B genes. The tachykinin peptides substance P (SP) and neurokinin A (NKA) are produced from the preprotachykinin A gene, whereas the preprotachykinin B gene encodes the precursor of the peptide neurokinin B (Hiroyuki et al., 1983).

2. Tachykinin receptors

The presence of multiple tachykinins and the fact that they have different actions suggest the existence of multiple tachykinin receptors. Three types of tachykinin receptors have been found: neurokinin-1 (NK1), neurokinin-2 (NK2), neurokinin (NK3) (Helke et al., 1990; Lee et al., 1986; Mantyh et al., 1989). Based on the binding affinity, their endogenous ligands are assumed to be substance P, neurokinin A and neurokinin B, respectively. Evidence from both receptor autoradiographic and lesions studies indicates that both NK-1 and NK-3 receptors are located in intrinsic striatal neurons whereas in the substantia nigra NK-3 receptors are located in dopamine neurons (Stoessl and Hill, 1990; Whitty et al., 1993). NK-2 receptors are widely distributed in the peripheral nervous system (Maggi, 1994). In the striatum, substance P (NK1) receptors are expressed by a large number of cholinergic interneurons (Gerfen, 1991; Kawaguchi et al., 1995). Activation of NK1 receptors in the striatum elicited a dose-dependent increase in acetylcholine release (Anderson et al., 1993, Guevara et al., 1993).

3. Tachykinin peptides and locomotor activity

The tachykinin peptides SP and NKA are located in the direct striatonigral pathway. Both *in vivo* and *in vitro* studies indicate that exogenous SP and NKA can elicit dopamine release from substantia nigra and striatum (Baruch et al., 1986; Pettit and Glowinski, 1986; Hokfelt et al., 1991), suggesting that both neuropeptides may act as a neurotransmitter in the direct striatonigral pathway. Although there is a prominent SP and NKA containing projection from the striatum to the substantia nigra (Bolam et al., 1990; Chang et al., 1988), the substantia nigra is devoid of NK1 and NK2 receptors (Mantyh et al., 1984; Stoessl and Hill, 1990). Thus the endogenous SP and NKA probably act on the NK3 receptors expressed on the midbrain dopamine neurons to regulate dopamine release.

Dopaminergic Neurotransmission and Behavioral Sensitization

1. Dopamine release in the terminal fields

Psychomotorstimulants are indirect dopamine releasing agents. The acute motor stimulation effect seems to be correlated with an increase in dopamine transmission in the dopamine terminal fields: NAc and dCPu. Thus, it is reasonable to assume that the sensitized behavioral responses are due to a further enhancement in dopamine release. *In vitro* studies found that psychomotorstimulant administration increased dopamine release in striatal and accumbal tissue slices or synaptosomes obtained from animals previously exposed to psychomotorstimulants (Robinson and Becker, 1982; Kolta et al., 1985; Castaneda et al., 1988; Yamada et al., 1988; Kalivas and Duffy, 1988). While *in*

vivo microdialysis studies demonstrate the similar result: dopamine release is augmented in the caudate-putamen and the nucleus accumbens in animals challenged with psychostimulants after repeated psychomotorstimulant treatment (Robinson et al., 1988; Akimoto et al., 1989; Kalivas and Duffy, 1990; Pettit et al., 1990). However, Several other studies found that dopamine release in response to a challenge injection after repeated psychomotorstimulant treatment was not enhanced compared with animals receiving a single dose of psychomotorstimulant injection (Hurd et al., 1989; Segal and Kuczenski, 1990a and 1990b; Kalivas and Duffy, 1993a).

2. Changes in the dopaminergic receptors

One hypothesis for the possible mechanism underlying behavioral sensitization to psychomotorstimulants is the desensitization of dopamine autoreceptors, causing augmentation in dopamine release after repeated psychomotorstimulant injections. Studies on animal models found that repeated administration of psychomotorstimulants readily induced subsensitivity of dopamine autoreceptors in the VTA and SNc (Henry et al., 1989; White et al., 1993). The hyposensitivity of autoreceptors was observed in both systemic and local iontophoretic administration of dopamine and dopamine agonists into the VTA and the SNc (Ackerman and White, 1991; White et al., 1993). However, subsensitivity of dopamine autoreceptors in the VTA and the SNc are transient, it was only observed during early withdrawal from repeated psychomotorstimulant treatment (White et al., 1993). Since behavioral sensitization is a long lasting

phenomenon, thus subsensitivity of the autoreceptors in the VTA and the SNc is not likely the mechanism underlying sensitization. Studies on the sensitivities of the postsynaptic D1 dopamine receptors in the nucleus accumbens and caudate putamen revealed that D1 dopamine receptors are hypersensitive in animals that have received a challenge injection following repeated psychomotorstimulant administration (White et al., 1993). Moreover, the period of supersensitivity of D1 dopamine receptors parallels the behavioral response to psychomotorstimulant challenge (White et al., 1993), suggesting that D1 dopamine receptor hypersensitivity may be related to behavioral sensitization.

3. Changes in dopamine reuptake

Dopamine transporter molecules take up the bulk of dopamine released into the synaptic clefts on dopamine terminals for recycling. Thus, the activity of the dopamine transporter molecules also affects the extracellular dopamine concentration. Previous studies showed that the effects of repeated psychomotorstimulants on the activity of dopamine transporter molecules have been inconsistent (Izenwasser and Cox, 1992; Peris et al., 1990; Yi and Johnson, 1990). The binding affinity of dopamine transporters showed increased, decreased and no change after repeated cocaine treatment (Farfel, et al., 1992; Alburges et al., 1993; Yi and Johnson, 1990). Ng and colleague demonstrated that there was a significant increase in dopamine reuptake 24 hours after discontinuing daily cocaine treatment, but not at 10 or 14 days of withdrawal from the repeated cocaine treatment (Ng et al., 1991; Parsons et al., 1991). However, *in vitro* studies on striatal

tissue slices revealed that the rate of dopamine uptake increased at 1, 14 and 21 days of withdrawal of cocaine self-administration (Meiergerd et al., 1994a, 1994b). The inconsistency in dopamine uptake during withdrawal periods following repeated cocaine treatment may explain the contradictory results of dopamine overflow in animals sensitized to psychomotorstimulants.

Excitatory Amino Acid (EAA) Transmission and Behavioral Sensitization

1. Glutamate synthesis, storage, release and metabolism

Glutamate is synthesized in neural tissues by two major pathways: 1) From a-ketoglutarate and donor amino acids by means of transamination, and 2) From glutamine through the action of glutaminase. The synthesized glutamate is then taken up into the vesicles by means of transporter proteins similar to the storage process for dopamine (Maycox et al., 1990; Nicholls and Attwell, 1990). Both *in vivo* and *in vitro* studies demonstrate that release of glutamate from the nerve terminals is a calcium dependent process probably caused by an exocytotic mechanism (Nicholls, 1989). Termination of the action of the released glutamate is carried out through the membrane transporter molecules.

2. Major excitatory amino acid afferents in the basal ganglia

Corticostriatal pathway

The cerebral cortex projects to the adjacent parts of the striatum; this projection has been demonstrated in the rat and other species (Webster, 1961; Carman et al., 1963, 1965; Cowan and Powell, 1966; Kitai, 1981; Gerfen, 1984; Royce, 1982; Wise and Jones, 1977). Despite this general topographic connection in the corticostriatal pathways, a more complicated picture of the corticostriatal projections has developed in recent years. The cortical fibers are remapped in the striatum by being broken up into local modules, and the divergence then is followed by reconvergence onto sets of basal ganglia output cells in the GP (Graybiel, 1994). Moreover, it was reported that the prefrontal cortex projects through the entire length of the striatum (Goldman and Nauta, 1977). The corticostriatal projections utilize the excitatory amino acid glutamate as neurotransmitter (Rebio et al., 1979; Fonnum et al., 1981). Stimulation of the cortex in intact animals evokes large amplitude excitatory postsynaptic potential (EPSPs) in medium spiny neurons of the neostriatum. The excitatory postsynaptic potential EPSP amplitude is correspondingly altered with stimulus intensity (Wilson et al., 1982, Calabresi et al., 1990). In addition, the prefrontal cortex also innervates other parts of the basal ganglia, including the nucleus accumbens, the ventral tagmental area and the substantia nigra (Beckstead, 1979; Gerfen et al., 1982).

Thalamostriatal pathway

The thalamostriatal input is another major afferent of the striatum. These projections originate mainly from the intralaminar nuclei of the thalamus

(Beckstead, 1984; Jayraman, 1984). Similar to the afferent from the cortex, the thalamostriatal fibers are topographically organized (Van der Kooy, 1979; Veening et al., 1980). Thalamostriatal terminals release the excitatory amino acid glutamate. Thus, simulation of the thalamus in intact animals produces EPSPs, an effect that is very similar to those of cortical stimulation (Wilson, 1983).

Amygdalostriatal pathway

Amygdalostriatal projections innervate not only the nucleus accumbens (Krettek and Price, 1978), but also the caudate-putamen (Royce, 1978; Russchen and Price, 1984). The organization of amygdalostriatal innervation is topographic. Caudal parts project the anteriomedial portion of the striatum, whereas rostral parts innervate the caudal and lateral division of the striatum. Some of the amygdalostriatal projections also use glutamate as a neurotransmitter (Fuller et al., 1984).

3. Evidence for the involvement of excitatory amino acid receptors in behavioral sensitization

The involvement of the excitatory amino acids in behavioral sensitization to psychomotorstimulants is indicated by the fact that coadministration of NMDA and non-NMDA receptor antagonists blocks the initiation and expression of behavioral sensitization (Karler et al., 1989; 1991; Wolf and Khansa, 1991; Stewart and Druhan, 1993). Much of the studies on behavioral sensitization have focus on the VTA, because repeated microinjection of amphetamine into the VTA produces

behavioral sensitization to a systemic challenge dose of amphetamine or cocaine (Kalivas and Weber, 1988; Vezina and Stewart, 1990; Hooks et al., 1992). Moreover, blockade of NMDA receptors in the VTA prevents the initiation of behavioral sensitization (Kalivas and Alesdater, 1993). Dopamine neurons in the VTA receive excitatory amino acid afferents from the prefrontal cortex (Carter, 1982). Stimulation of these projections evokes burst-firing patterns in the VTA dopamine neurons (Gariano and Groves, 1988). The burst firing results from the stimulation of the NMDA receptors (Overton and Clark, 1992; Johnson et al., 1992). Increases in the firing rates of dopamine neurons may lead to an elevation in axonal dopamine release per action potential, compared with pacemaker-like firing patterns (Gonon, 1988; Suaud-Chagny et al., 1992).

In addition to the EAA projection from the prefrontal cortex to the VTA, other EAA afferents to the basal ganglia may also play a role in behavioral sensitization. Indeed, destruction of the EAA innervation from the hippocampus to the nucleus accumbens attenuates behavioral sensitization to repeated administration of amphetamine (Yoshikawa et al., 1991). The amygdala also has an EAA projection to the nucleus accumbens and striatum (Kelley et al., 1982; Mogenson and Yang, 1991). Blockade of NMDA receptors in the amygdala prevents behavioral sensitization (Kalivas and Alesdatter, 1993). These findings suggest those EAA inputs from both hippocampus and amygdala to the NAc are critical in the expression of behavioral sensitization to psychomotorstimulants.

The Goal of This Thesis

Repeated administration of psychomotorstimulants causes long-term changes in behavior ranging from addiction to behavioral sensitization. Many of these behaviors depend on the nigrastriatal and mesolimbic systems of the basal ganglia. Although converging evidence suggests that dopamine neurotransmission is involved in the behavioral alterations in response to repeated exposures to psychostimulants, the mechanisms underlying behavioral sensitization remains largely unknown.

The objective of this thesis is to explore the neuronal substrates of behavioral sensitization induced by the psychomotorstimulants methamphetamine and cocaine. The following procedures are used: 1) Examine the mRNA levels of tyrosine hydroxylase during the psychomotorstimulant treatment and withdrawal. 2) Determine if striatal neuropeptide mRNA levels were changed by repeated psychomotorstimulant administration. 3) Address the role of neurotransmitter dopamine in behavioral sensitization to psychomotorstimulants. 4) Measure glutamate levels in the basal ganglia of sensitized animals.

Aim #1 Are tyrosine hydroxylase (TH) mRNA levels changed after repeated METH treatment and during withdrawal?

To see whether tyrosine hydroxylase mRNA levels change after repeated METH treatment and during METH withdrawal period, rats were treated with METH (4 mg/kg) twice daily for 6 consecutive days. One group was sacrificed on the same day of drug injection, while another group was sacrificed 14 days after the last METH injection. TH mRNA levels in both the VTA and SNc was measured by *in situ* hybridization histochemistry.

Aim #2 Do striatal preprotachykinin and preproenkephalin mRNA levels change in response to repeated METH administration?

In order to establish whether striatal tachykinin and enkephalin peptides might be involved in psychomotorstimulant induced behavioral sensitization, the mRNA levels of these peptide precursors were examined by *in situ* hybridization. PPT and PPE mRNAs were measured at 1, 3 and 6 days of repeated METH injections. The mRNA levels for PPT and PPE were compared between time points.

Aim #3 Are extracellular dopamine levels altered by psychomotorstimulant exposure?

To determine whether dopamine plays a role in behavioral sensitization, extracellular dopamine was measured in the NAc, dCPu, SNc and VTA at day 4 of withdrawal following 7 daily injections of psychomotorstimulant treatment. The results were compared to dopamine release in response to acute injection.

Aim #4 Does glutamate play a role in behavioral sensitization to psychomotorstimulants?

To determine whether glutamate plays a role in sensitization to psychomotorstimulants, extracellular glutamate was examined in the NAc, dCPu, SNc and VTA of rat 3 days after 7 daily psychomotorstimulant injections (day 4 of withdrawal). The results were compared with glutamate released in response to acute injection of the same drug.

CHAPTER II

Contrasting Effects of Repeated Treatment Vs. Withdrawal of Methamphetamine on Tyrosine Hydroxylase Messenger RNA Levels in the Ventral Tegmental Area and Substantia Nigra Compacta of the Rat Brain

INTRODUCTION

Locomotor activity and exploratory behaviors are subserved by dopaminergic pathways whose cell body areas reside in the mesencephalon and express tyrosine hydroxylase (TH), the rate-limiting enzyme of cathecholamine biosynthesis (Kalivas, 1985; Schalling et al., 1986). Dopaminergic perikarya of the nigrostriatal projection are located in the substantia nigra compacta (SNc) (Bjorklund and Lindvall, 1984; Fallon and Moore, 1978), while mesolimbic and mesocortical pathways originate from dopaminergic perikarya of the ventral tegmental area (VTA) (Hokfelt et al., 1984; Loughlin and Fallon, 1984; Swanson, 1982). Repeated microinjections of amphetamine into the SN or VTA made animals more responsive to the locomotor-enhancing properties of a subsequent challenge injection of the drug, thus implicating dopaminergic perikarya of the mesencephalon in the development of behavioral sensitization to this class of psychomotor stimulant (Joyce and Iversen, 1979; Kalivas and Weber, 1988). Moreover, pretreatment with psychomotor stimulants results in intensified drug-stimulated release of dopamine from dopaminergic terminal fields of the neostriatum as well as increased locomotor responses when animals are reexposed to the drug several days after cessation of treatment (Akimoto et al., 1989).

In addition to the classical transmitter dopamine, a substantial population of dopaminergic neurons of the SNc and VTA colocalize the neuropeptide cholecystokinin (CCK) with tyrosine hydroxylase (Kiyama et al., 1991; Schalling et

al., 1990). Cholecystokinin-dopamine interactions have been documented in dopaminergic cell body areas of the mesencephalon as well as in terminal fields of the nucleus accumbens (Crawley, 1991). For example, microinjection of physiological doses of cholecystokinin into the nucleus accumbens potentiates dopamine-induced hyperlocomotion (Crawley et al., 1985). In the midbrain, CCK is postulated to amplify the inhibitory effect of dendritically released dopamine at D2 autoreceptors located on dendrites and soma of dopaminergic neurons (Crawley, 1989; Freeman et al., 1991). Since dopaminergic parameters are implicated in the phenomenon of sensitization to drugs of abuse such as methamphetamine (Kalivas and Stewart, 1991; Kalivas, 1993), we have evaluated tyrosine hydroxylase and preprocholecystokinin (PPCCK) mRNA levels in midbrain SNc and VTA by in situ hybridization histochemistry in rodents that received repeated injections of methamphetamine and were sacrificed either 5 h or 15 days after the treatment.

METHODS

Animals and drug treatment

Male Sprague-Dawley rats (Charles River, New York, NY) weighing approximately 200 g at the start of the experiment were maintained in a temperature-controlled environment under a 14:10 h light/dark cycle with free access to food and water. Rats (n = 8 per group) were injected intraperitoneally twice daily for 5 consecutive days with methamphetamine (METH) (4 mg/kg of

body weight dissolved in phosphate-buffered saline, pH 7.4) at 1000 and 1500 h. On the sixth day, rats received an injection of METH at 1000 h and were sacrificed at 1500 h. A separate group of animals received METH and MK-801 (0.1 mg/kg) concurrently. To assess the effect of withdrawal, a set of rats was treated as above and sacrificed at 1500 h 15 days after the last injection. Control rats received 0.5 ml of phosphate-buffered saline, pH 7.4.

Radiolabeling of synthetic oligonucleotide probes

Hybridization probes were labeled at the 3' end by terminal deoxynucleotidyl transferase essentially as described (Angulo, 1992). The reaction consisted of 100 mM potassium cacodylate buffer (pH 7.2), 25 mM cobalt chloride, 0.2 mM dithiothreitol (DTT), 70 uCi of ^{35}S-dATP (specific activity of >1,000 Ci/mmol; Amersham, Arlington Heights, IL), 0.25 ug of probe, and 12 U of terminal deoxynucleotidyl transferase (total reaction volume of 10 ul). The reaction mixture was incubated at 37°C for 1 h, and the oligonucleotide probe was separated from unincorporated isotope by chromatography on a Sephadex G25 Quick Spin column (Boehringer/Mannheim, Indianapolis, IN) at room temperature. The column buffer consisted of 10 mM Tris-Cl buffer (pH 8.0) and 1 mM EDTA.

Hybridization probes

TH and PPCCK mRNAs were detected with antisense synthetic oligonucleotide probes. Probes were screened with GENBANK and found not to cross-hybridize with known nucleic acid sequences. The following probes and

sequences were used: TH mRNA, 5'dTCAAAGGCTCGGACCTCAGGCTCCTCTGAC corresponding to nucleotides 1,223-1,252 of the cloned mRNA sequence (Grima et al., 1985); and PPCCK mRNA, 5'dCCATCCAGCCCATGTAGTCCCGGTCACTTATCCTGTGGCTAG corresponding to nucleotides 6,738-6,780 of the rat gene (Deschenes et al., 1985). The specificity of the hybridization probes was determined as already described (Angulo et al., 1990). TH and PPCCK probes displayed signal: noise ratios of 18 and 11, respectively.

Localization of mRNA by in situ hybridization histochemistry

Animals were decapitated and the brains frozen on powdered dry ice. Coronal sections (20 um in thickness) were cut in the cryostat at - 15°C and thermally mounted onto gelatin-coated glass. The tissue was fixed for 30 min at 4°C in 2% paraformaldehyde 0. 1 M sodium phosphate buffer (pH 7.2) and washed for 1-2 min in 0.5 x SSC buffer (1 x SSC = 0.15 M sodium chioridel/0.015 M sodium citrate buffer, pH 7.0). Sections (three per slide) were air-dried at room temperature (RT), rinsed for 1 min (RT) in acetylation buffer (0.1 M triethanolamine, pH 8.0), and then acetylated for 10 min (RT) with 0.25% acetic anhydride dissolved in acetylation buffer. Slides were washed twice in 2 x SSC for 5 and 2 min and finally air-dried. Sections were hybridized overnight at 37°C in a humidified environment with a solution consisting of 0.2% (w/v) bovine serum albumin, 0.1% (w/v) polyvinylpyrrolidone, 0.2% (w/v) ficoll, 3 x SSC buffer, 50% formamide, 10% dextran sulfate, 10 mM dithiothreitol (DTT), 100 ug/ml of

sheared and denatured salmon sperm DNA, 400 ug/ml of tRNA, 1 mM EDTA, 4 ug/ml of heparin, and 6-8 *x* 106 cpm/ml oligonucleotide probe (12-16 ng/ml). The hybridization solution was applied in a volume of 100 ul, and the slide was coverslipped. The next day, coverslips were removed by dipping in 2 x SSC buffer (RT). Sections were washed (RT) three times for 20 min each in 1 *x* SSC buffer/i mM DTT followed by two washes (20 mm each) at 45°C in 1 x SSC/1 mM DTT. Then came one final wash for 20 min at 45°C in 1x SSC. The sections were dehydrated in successive alcohol solutions (50% and 85%/0.3 M ammonium acetate; 100% ethanol) and dried with a fan. Slides were apposed to Hyperfilm MP (Amersham) for 15 days (TH mRNA) and 21 days (PPCCK mRNA).

Quantification of mRNA levels

Messenger RNA levels were determined by quantifying grey levels on x-ray autoradiograms (Hyperfilm MP; Amersham) in regions corresponding to the SNc and VTA as shown in Figure 2. Tissue sections were stained with cresyl violet, and only sections histologically equivalent between control and treatment groups were used for the mRNA analysis. Grey levels were quantified with an image analysis system utilizing the NIH Image 1.49 software VDM (Rasband, 1993). Five sections per animal were bilaterally quantified and the values averaged to generate an optical density value that corresponds to mRNA abundance per animal. Each treatment group (including control) consisted of eight animals; thus optical density values

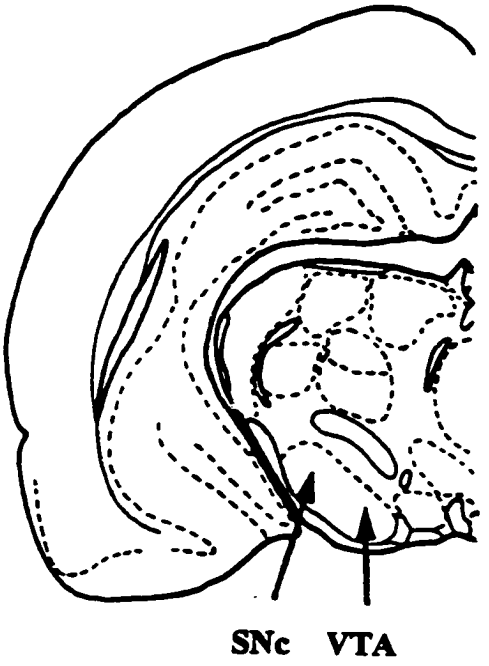


Figure 2. Diagrammatic representation of the midbrain in coronal section at the level of the substantia nigra compacta (SNc) and the ventral tegmental area (VTA) of the rat brain. The plane of section corresponds to plate 47 of A stereotaxic Atlas of the Rat Brain (Pellegrino et al., 1979).

from each animal were averaged and the value obtained taken as representative of mRNA abundance. Statistical analysis paired values from control with drug treatment groups by ANOVA.

RESULTS

Effect of methamphetamine treatment on tyrosine hydroxylase mRNA levels

Daily intraperitoneal injections of methamphetamine (4 mg/kg) for 6 consecutive days failed to significantly affect TH mRNA levels in the VTA 5 h after the last injection (Fig. 3A). In contrast, TH mRNA levels were significantly reduced 25% below controls in the SNc (Fig. 3A). Concurrent administration of METH with the N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 potentiated the effect of METH in the SNc but had no effect in the VTA (Fig. 3A). Chronic treatment with MK-801 alone did not cause significant changes of TH mRNA levels in the substantia nigra compacta or ventral tegmental area of the rat brain (unpublished results).

In order to assess neurochemical effects of methamphetamine withdrawal, a separate set of rats consisting of three groups (control, METH, and METH/MK-801; eight male rats per group) was treated as above but sacrificed 15 days after the last METH injection. Tyrosine hydroxylase mRNA levels were increased 42% relative to controls in the VTA of animals withdrawn from METH. Coadministration of MK-801 with METH prevented the increase (Fig. 3B). METH withdrawal for 15 days did not affect TH mRNA levels in the SNc (Fig. 3B).

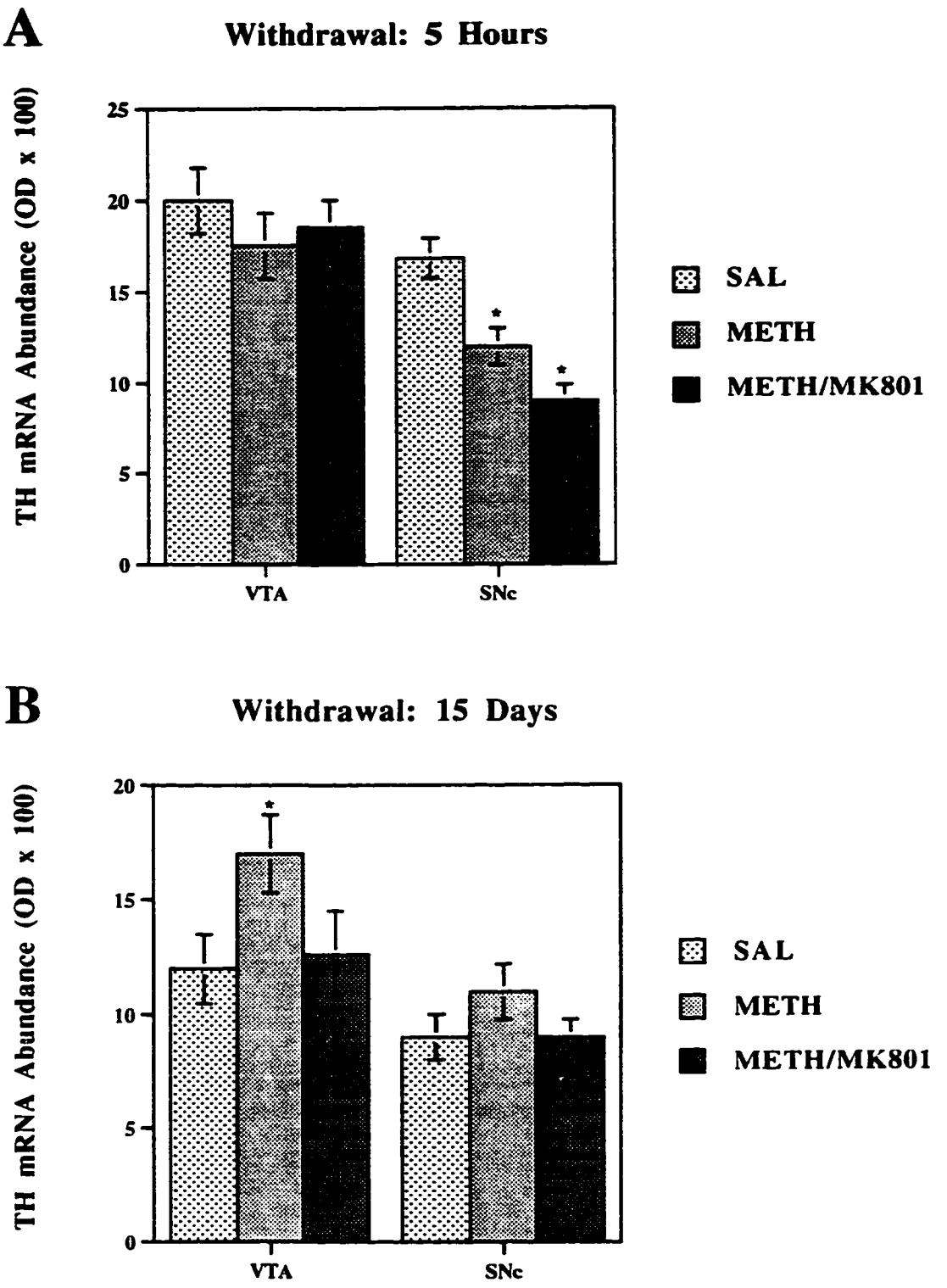


Figure 3. Tyrosine hydroxylase (TH) mRNA levels at the level of the substantia nigra zona compacta (SNc) and the ventral tegmental area (VTA). TH mRNA was detected in coronal sections of brain tissue by in situ hybridizatioon histochemistry. Rats were injected (ip) for 6 consecutive days with methamphetamine (METH) (4 mg/kg) and sacrificed 5 h (A) or 15 days (B) after the last injection. Controls (SAL) were injected with phosphate-buffered saline, pH 7.4. A group of animals (eight) received METH and MK-801 (0.1 mg/kg) concurrently. Symbols over the bars represent standard error of the mean (SEM) values. Bar values represent optical density values obtained from x-ray film autoradiograms (eight animals per treatment group). *$p < 0.05$ (Student's t-test). (see Synapse (1996) 24, 220)

Effect of methamphetamine treatment on preprocholecystokinin mRNA levels

Preprocholecystokinin (PPCCK) mRNA was detected in coronal sections of brain tissue from the same animals used for TH mRNA analysis. In contrast to the effects of METH treatment and withdrawal on TH mRNA levels in the SNc and VTA, PPCCK mRNA was not affected either 5 h (Fig. 4A) or 15 days (Fig. 4B) after METH treatment in these brain regions. PPCCK mRNA levels were increased 11% in the VTA of animals withdrawn from METH, and this increase was prevented by concurrent administration of MK-801 (Fig. 4B); however, the increase did not approach statistical significance relative to controls at the 95% confidence level (Student's t-test). A similar trend was observed in the SNc of animals withdrawn from methamphetamine for 15 days (Fig. 4B).

DISCUSSION

In the present study we utilized a drug treatment paradigm that is known to result in behavioral sensitization to amphetamine (Wolf et al., 1994) and assessed TH mRNA levels 5 h after the last injection or at day 15 of abstinence. We found the messenger RNA coding for the catecholamine rate-limiting enzyme tyrosine hydroxylase elevated in the VTA 15 days after pretreatment with methamphetamine. Conversely, TH mRNA abundance in the SNc was found decreased 5 h after repeated treatment. The latter may result from negative feedback mediated by dendritic dopamine autoreceptors (Bjorklund and Lindvall, 1975).

Repeated treatment with amphetamine produces a form of behavioral

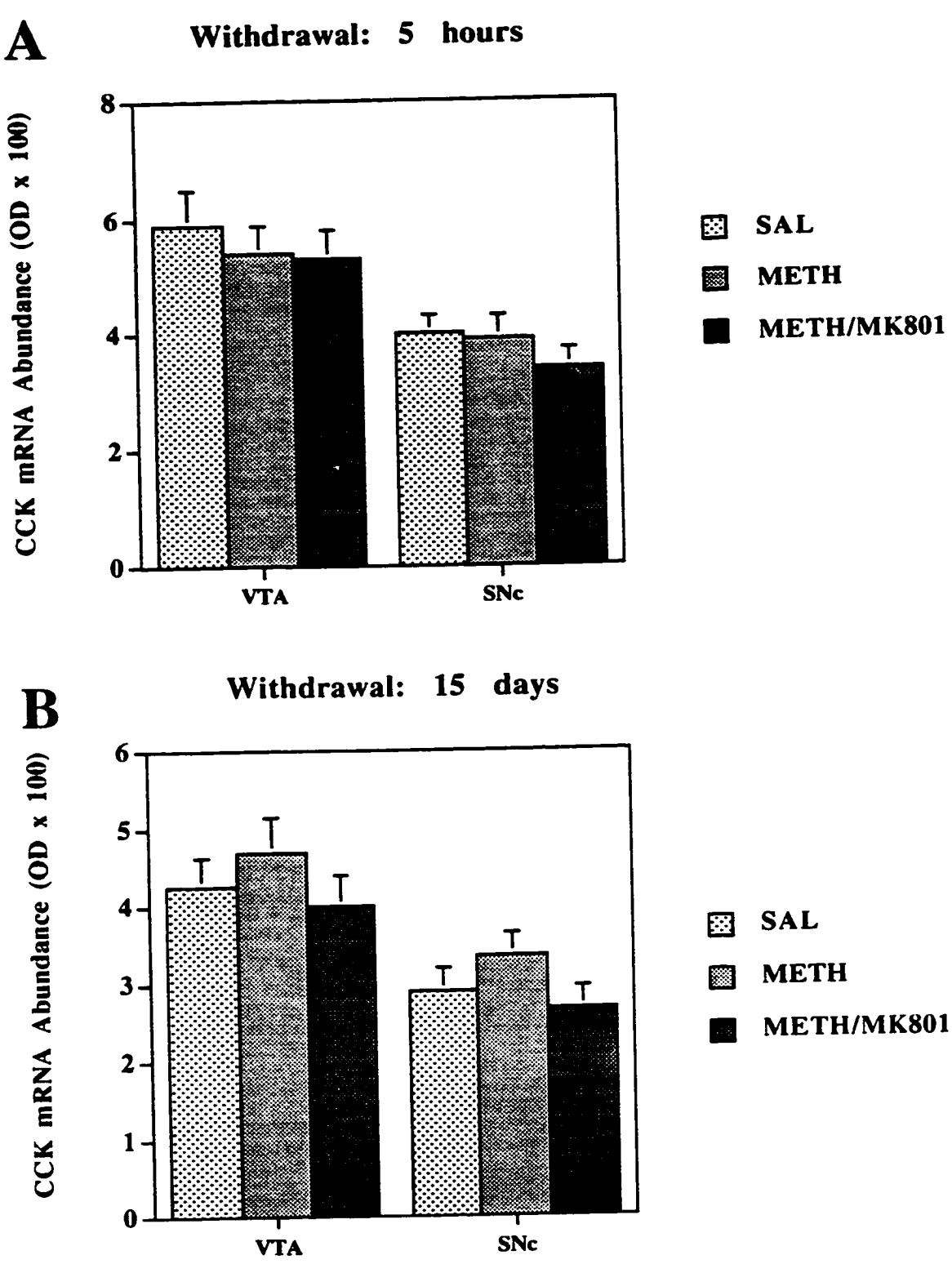


Figure 4. Preprocholecystokinin mRNA levels in the substantia nigra zona compacta (SNc) and ventral tegmental area (VTA) from controls (SAL) and (METH) (4 mg/kg) treatment animals. Messenger RNA was detected in coronal sections of brain tissue by *in situ* hybridization histochemistry. Bar values reperesent optical density values from x-ray film autoradiogram. The noncompetitive NMDA receptor antagonist MK-801 was coadministration with METH at a dose of 0.1 mg/kg of body weight. $*p < 0.05$ (paired Student's *t*-test). (See Synapse (1996) 24,221)

sensitization that persists for several weeks after withdrawal from the drug (Robinson and Becker, 1986). For example, a drug treatment paradigm involving injections of amphetamine twice daily (5 mg/kg, ip) for 5 consecutive days resulted in significant increases of stereotypic behaviors upon reexposure to amphetamine 15 and 30 days after treatment, which may be accounted for by augmented activity of the nigrostriatal dopaminergic system in psychostimulant pretreated animals (Kolta et al., 1985). Moreover, amphetamine stimulated more dopamine release from striatal slices of pretreated rats than from saline-injected controls (Kolta et al., 1985). These observations suggest that dopaminergic transmission is augmented by psychostimulant pretreatment in the basal ganglia of the rodent brain.

Augmentation of dopamine release by psychostimulant pretreated animals upon exposure to a challenge dose of amphetamine requires a time interval of at least 15 days of withdrawal from amphetamines (Paulson et al., 1991). For example, dopamine release from dorsal striatum and nucleus accumbens evoked by a challenge dose of amphetamine from animals that had been pretreated with an escalating amphetamine schedule was observed 15 and 28 days (Paulson and Robinson, 1995; Robinson et al., 1988). Our results demonstrate that TH mRNA levels are elevated in the ventral tegmental area 15 days after repeated METH treatment. Thus, our data are compatible with a model of enhanced dopaminergic activity at the level of TH mRNA which might reflect elevated levels of TH enzyme and activity as well.

The ventral tegmental area and its projections to the nucleus accumbens and prefrontal cortex have been implicated as drug reward regions mediating some

of the reinforcing properties of opiates and psychostimulants such as cocaine and amphetamines (Koob and Bloom, 1988; Wise and Bozarth, 1987). Rats chronically treated with cocaine or morphine and sacrificed within 16 h of the last injection displayed elevated levels of tyrosine hydroxylase in the VTA but not in the nucleus accumbens, caudate-putamen, or substantia nigra (Beitner-Johnson and Nestler, 1991). In the present study, we did not observe changes in TH mRNA levels in the VTA 5 h after repeated METH treatment, although it is conceivable that TH enzyme levels may be increased due to altered axonal transport since chronic cocaine and morphine have been shown to decrease the levels of neurofilament proteins in the VTA (Beitner-Johnson et al., 1992).

Concurrent administration of the noncompetitive NMDA receptor antagonist MK-801 with METH prevented the increase of TH mRNA in the VTA of rats subjected to 15 days of abstinence, suggesting that excitatory amino acid activity via NMDA receptors is required for the development of the enduring effects of METH treatment which may be associated with behavioral sensitization. For example, using a drug treatment and dosage paradigm that produces behavioral sensitization, coadministration of MK-801 with amphetamine effectively blocked amphetamine-induced autoreceptor subsensitivity to dopamine agonists in the VTA as well as dopamine D1 receptor supersensitivity in the nucleus accumbens (Ackerman and White, 1990; Henry and White, 1991; Wolf et al., 1994).

In conclusion, the results demonstrate that withdrawal from repeated treatment with methamphetamine alters tyrosine hydroxylase mRNA levels in peri-karya of mesolimbic and mesocortical neurons of the ventral tegmental area of the

rat brain. The increase in the TH mRNA level in the VTA was observed at day 15 of withdrawal and was prevented by concurrent pretreatment with the NMDA receptor antagonist MK801, suggesting a direct involvement of glutamatergic transmission in METH-induced alterations of the dopaminergic system. Work in progress will assess the levels and activity of TH utilizing the drug schedule paradigm employed in the present study.

CHAPTER III

Progressive Augmentation of Striatal and Accumbal Preprotachykinin mRNA Levels by ChronicTreatments with Methamphetamine and Effect of Concurrent Administration of the N-methy-D-aspartate Receptor Antagonist MK-801

INTRODUCTION

The caudate-putamen and nucleus accumbens are major relay centers of the basal ganglia (Gerfen, 1992). Dopaminergic perikarya of the mesenscephalon represent the main source of dopaminergic input to the neostiatum. Cell bodies of the substantia nigra compacta innervate the caudate-putamen giving rise to the nigrostriatal pathway, while dopaminergic neurons of the ventral tegmental area project to the accumbens and prefrontal cortex and these projections are referred to as mesolimbic and mesocortical pathways, respectively (Bjorklund and Lindvall, 1984; Fallon and Moore, 1978). The dopaminergic innervation of the neostriatum has been implicated in numerous behaviors and has been shown to modify the activity and the neurochemical plasticity of neostriatal projection neurons (Le Moal and Simon, 1991; Angulo and McEwen, 1994). Pharmacological and deafferentation studies have established that mesencephalic dopaminergic transmission exerts tonic inhibition on neostriatal enkephalinergic and neurotensinergic expression, while tonically stimulating tachykinin and dynorphin systems at both peptide and mRNA levels (Angulo, 1992; Bannon et al., 1986; Hong et al., 1980; Li et al., 1990; Gerfen et al., 1991; Angulo and McEwen, 1994). Striatal neuropeptides, in turn, can influence the function of dopaminergic neurons. Microinjection of neuropeptides or protease-resistant peptide analogs into the midbrain ventral tegmental area affects dopamine metabolism in terminal field areas of the nucleus accumbens (Kalivas, 1985). Thus, striatal and accumbal neuropeptides might play an important role as modulators of dopamine-dependent

functions and behaviors, such as sensitization to psychomotor stimulants of abuse like cocaine and amphetamine.

Neuropeptides might affect some behaviors by modulating dopaminergic activity in the brain. Neurochemical investigations demonstrate that neuropeptides affect the metabolism of dopamine in terminal fields areas of the forebrain (Kalivas, 1985). Direct application of neuropeptides into the dopaminergic cell body groups of the mesencephalon results in locomotor responses. For example, direct application of the tachykinin peptide substance P onto A10 dopaminergic neurons increased locomotion and rearing and decreased grooming (Stinus et al., 1978; Kelly et al., 1985). A related tachykinin peptide, neurokinin A, had similar effects as substance P, but was more potent (Kalivas, 1985). Direct application of opioid peptides onto A10 dopaminergic neurons produced behavioral sensitization of motor activity to subsequent application of peptidase-resistant analogs of enkephalin (Kalivas and Stewart, 1991). The foregoing observations suggest that neuropeptides might play an important role in the regulation of locomotor activity, as well as in the locomotor-enhancing properties of psychostimulant drugs.

In the light of these observations, we have evaluated the time course of repeated treatment with methamphetamine (METH) up to 6 days, as well as withdrawal for 15 days, on the levels of preprotachykinin and preproenkephalin mRNAs in subdivisions of the caudate-putamen and the nucleus accumbens by in situ hybridization histochemistry. In addition, we have assessed the involvement of concurrent administration of methamphetamine with the non-competitive NMDA receptor antagonist MK-801. To assess the effect of withdrawal from METH on

neostriatal neuropeptide expression, rodents that were treated with METH for 6 consecutive days were sacrificed at day 15 of abstinence from the drug.

METHOD

Animals

Male Sprague-Dawley rats (Charles River, New York) weighing approximately 250-350g were housed in a temperature-controlled environment under a 14:10 h light / dark cycle with free access to food and water. Rats were allowed to acclimate for at least 1 week after arrival in the animal facility, followed by daily handling for several days prior to the experiments.

Radiolabeling of the Oligonucleotide Probes.

The probes were labeled at the 3' end by terminal deoxynucleotide transferase essentially as described (Angulo et al., 1990). The reaction consisted of 100mM potassium cacodylate buffer (pH 7.2), 25mM $CoCl_2$, 0.2 mM dithiothreitol (DTT), 70uCi of ^{35}S-dATP (Amersham, specific activity of > 1,000 Ci / mmol), 0.25 ug of probe, and 12 U of terminal deoxynucleotidyl transferase (total reaction volume of 10 ul). The reaction mixture were incubated at 37°C for 1 hour and the radiolabeled oligonucleotide probe was separated from unincorporated isotope by chromatography on a Sephadex G-25 Quick Spin column (Boehringer/Mannheim) at room temperature. The column buffer consisted of 10mM Tris-Cl buffer (pH 8.0) and 1mM EDTA.

Hybridization probes

Preproenkephalin (PPE) and preprotachykinin (PPT) mRNAs were detected with antisense synthetic oligonucleotide probes. Probes were screened with GENBANK and found not cross-hybridize with known nucleic acid sequences. The specificity of the probes was determined as already described (Angulo et al., 1990). The following probes and sequences were used: PPE mRNA, 5'dCAAGTCGTCCTCATCCTGTTTGCTGC-TGCT and 5'dCTCCACGGGGTAAAGCTCATCCATCTTCTT corresponding to amino acids 86-95 and 112-121 of the rat prohormone (Rosen et al., 1984); and PPT mRNA, 5'dCATTAATCCAAAGAACTGCTGAGGCTTGGGTCT and 5'dGCCCATTAGTCCAACAAAGGAATCTGTTTTATG corresponding to amino acids 58-68 and 83-93 of the rat prohormone (Krause et al., 1987).

Localization of mRNA by in situ hybridization histochemistry.

Rats were decapitated and the brains were frozen on powdered dry ice. Coronal sections were cut in the cryostat at -17°C and mounted onto glass slides treated with vatabone. The slices were fixed for 30 min. at 4°C in 2% paraformaldehyde/0.1M sodium phosphate buffer (pH 7.2) and washed for 1-2 min in 0.5X SSC buffer (1X SSC = 0.15 M sodium chloride/0.015M sodium citrate, pH 7.0). Sections were air-dried at room temperature (RT), rinsed for 1 min. (RT) in acetylation buffer (0.1 M triethanolamine, pH 8.0) and then acetylated for 10 min. with 0.25% acetic anhydride dissolved in acetylation buffer at room

temperature. Slides were washed in 2X SSC for 5 and 2 min. and finally air-dried. Sections were hybridized overnight at 37°C in a humidified environment with a solution consisting of 0.2% (w/v) bovine serum albumin, 0.1% (w/v) polyvinylpyrrolidone, 0.2% (w/v) Ficoll, 3X SSC buffer, 50% formamide, 10% dextran sulfate, 10mM DTT, 100ug/ml of sheared and denatured salmon sperm DNA, 400 ug/ml of tRNA, 1mM EDTA, 4ug/ml of heparin and 6-8 x 10^4 cpm/ml oligonucleotide probe (12-16 ng/ml). The hybridization solution was applied in a volume of 100 ul per slide and the slides were coverslipped. The next day, coverslips were removed by dipping in 1X SSC buffer (RT). Sections were washed (RT) 3 times 20 min. each in 1X SSC buffer/1mM DTT followed by 2 washes 20 min. each at 45°C in 1X SSC/1mM DTT. Then, one final wash for 20 min. at 45°C in 1X SSC. Tissue was dehydrated in successive alcohol solutions (50%/0.3M ammonium acetate, 85%/0.3M ammonium acetate and 100% ethanol) and dried with a fan. Slides were exposed to Hyperfilm for seven days (PPE), 14 days (PPT) and 4 weeks (PPD).

Quantification of neuropeptide mRNA

Films were developed. Messenger RNA levels were determined by quantifying grey levels on the x-ray autoradiogram in regions corresponding to the caudate-putamen and nucleus accumbens (Fig. 5). Grey levels were quantified with an image analysis system utilizing the NIH Image 1.49 software VDM. Six sections

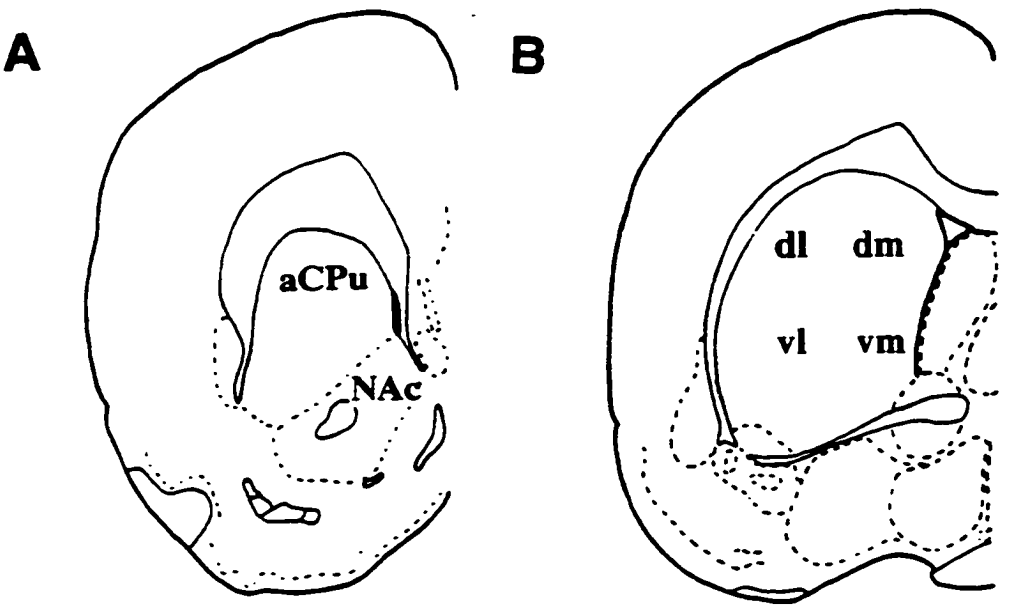


Figure 5. Schematic representation of subdivisions of the caudate-putamen and the nucleus accumbens in coronal sections. Panels A and B are adapted from *A stereotaxic Atlas of the Rat Brain* by Pellegrino *et al.* (1979) and correspond to plates 14 and 19, respectively. A. aCPu, anterior caudate-putamen; NAc, nucleus accumbens. B. dlCPu, dmCPu, vlCPu and vmCPu: dorsolateral, dorsomedial, ventrolateral and ventromedial aspects of the caudate-putamen, respectively.

per animals were bilaterally quantified and the values were averaged to generate an optical density value that represented that animal. There were eight animals in each group, the optical density values from eight animals were averaged and the mean value was taken as representative of mRNA level.

RESULTS

Effect of repeated methamphetamine treatment and withdrawal on preprotachykinin mRNA levels in the striatum and accumbens:

Acute treatment with METH (4 mg/kg) increased PPT mRNA levels in dorsal and ventral aspects of the caudate-putamen (CPu) but, paradoxically, concurrent administration of the NMDA receptor antagonist MK-801 attenuated the effect of METH in dorsomedial CPu (dmCPu) while potentiating in the ventromedial CPu (vmCPu) (Fig. 6A). At day 3 of treatment, statistically significant increases were observed in all subdivisions of the CPu, except ventrolateral CPu (vlCPu) and anterior CPu (aCPu) (Fig. 6B). Interestingly, MK-801 attenuated the effects of METH most effectively in the nucleus accumbens (Fig. 6B). Repeated treatment with METH for 6 consecutive day increased PPT mRNA abundance in all aspects of the caudate-putamen and the nucleus accumbens (NAc)(Fig. 6C). The largest increase relative to vehicle-injected controls was observed in medial aspects of the CPu: 72% and 96% in dorsomedial and ventromedial CPu, respectively (Fig. 6C). Dorsolateral and ventrolateral subdivisions displayed smaller

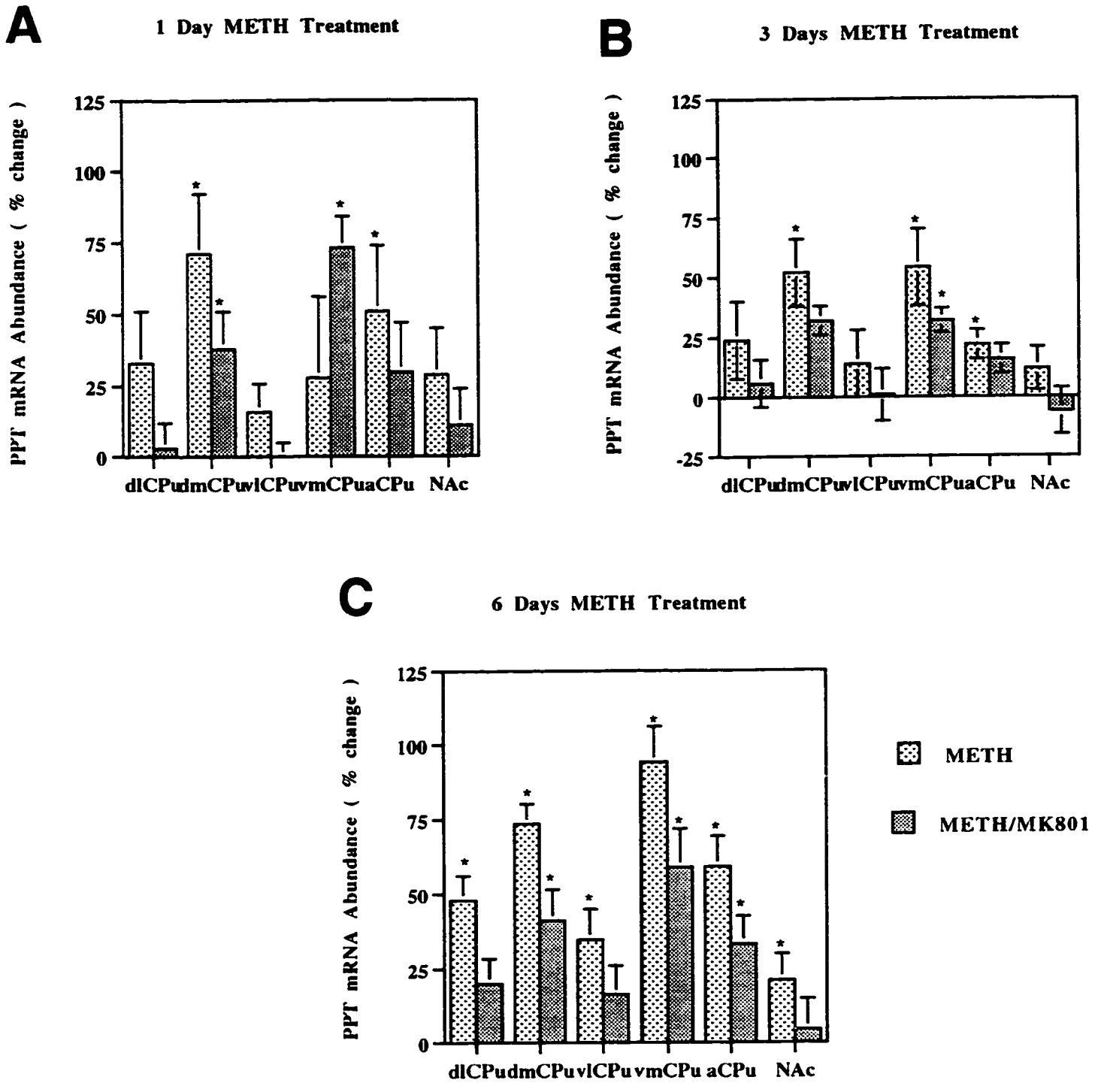


Figure 6. Time course of repeated treatment with methamphetamine on pre PPT mRNA was detected in coronal sections of brain tissue with synthetic oligonucleotide probes by in situ hybridization histochemistry. Messenger RNA levels were determined with image analysis system and the optical density values obtained expressed as percentage change relative to controls. Each time point consisted of 24 animals: eight received vehicle, eight received METH and the remaining eight received METH and MK-801, concurrently. Note the increase of PPT mRNA accumulation between days 3 and 6. Values over the bars represent SEM. NAc, nucleus accumben; aCPU, anterior caudate-putamen; dlCPU, dmCPU, vlCPU, vmCPU: dorsolateral, dorsomedial, ventrolateral and ventromedial aspects of the caudate-putamen, respectively. *$p < 0.05$ (paired Student's t-test).

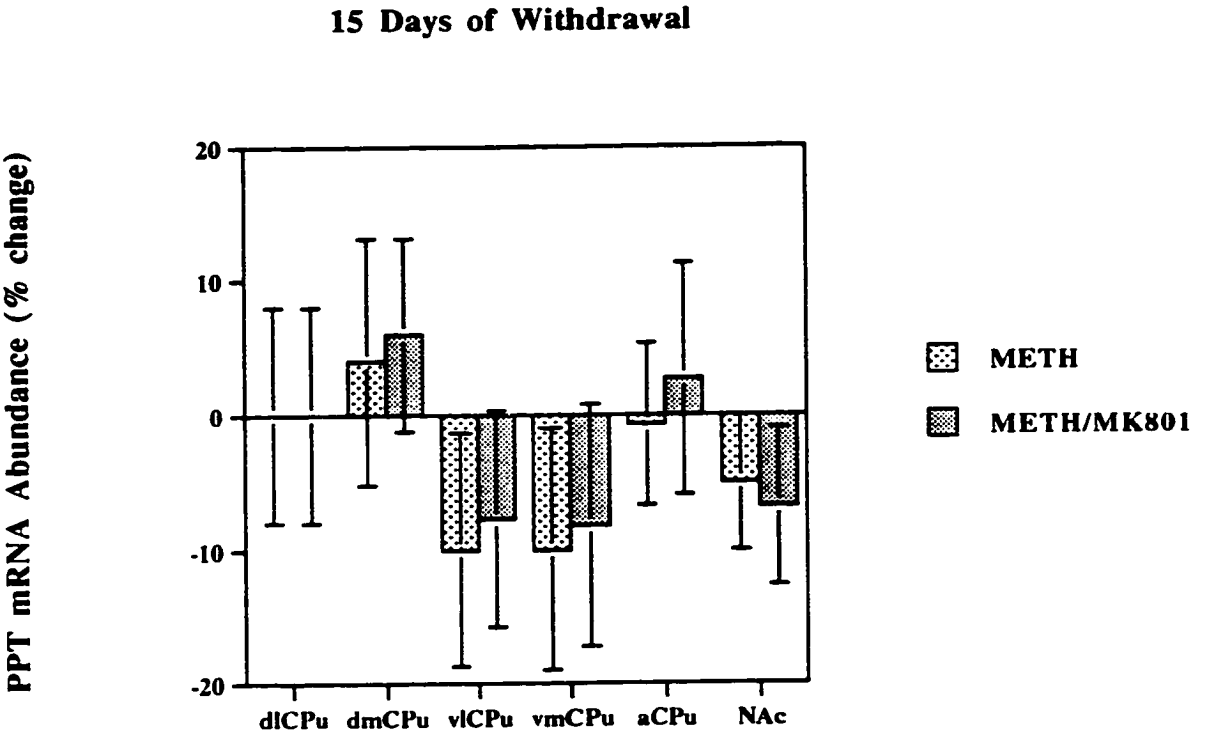


Figure 7. Effect of 15 days of withdrawal from METH treatment on PPT mRNA expression in the caudate-putamen and nucleus accumbens of the rat brain. PPT mRNA levels were assessed by in situ hybridization histochemistry in coronal sections of brain tissue. PPT mRNA levels were quantified with an image analysis system and the results are expressed as percentage change relative to control values. Rats (n=8 per treatment group) were treated with METH or METH/MK-801 and were sacrificed 15 days after the last injection. Positive values over the bars represent SEM values. NAc, nucleus accumbens; aCPu, anterior caudate-putamen; dlCPu, dmCPu, vlCPu and vmCPu: dorsolateral, dorsomedial, ventrolateral and ventromedial aspects of the caudate-putamen, respectively. $*p < 0.05$ (paired Student's *t*-test). (Published in Neuropharmacology, 36, 329)

Increases (47% and 35%, respectively; Fig. 6C). Anteriorly, PPT mRNA was increased 63% in aCPu and 21% in the accumbens, respectively, relative to vehicle-injected controls (Fig. 6C). Concurrent administration of the non-competitive NMDA receptor antagonist MK-801 with METH attenuated METH-induced elevations of PPT mRNA in all aspects of the CPu and the NAc (Fig. 6C).

In order to determine if the increased levels of neostriatal PPT mRNA induced by repeated injections of METH persisted after cessation of treatment, rodents that had been treated consecutively with METH for 6 days were withdrawn from the drug for 15 days. At day 15 of withdrawal, PPT mRNA levels in all aspects of the CPu, as well as the NAc, were statistically indistinguishable from vehicle-injected controls at the 95% confidence level (Fig. 7). Similarly, concurrent treatment with MK-801 did not affect PPT mRNA levels in the neostriatum at day 15 of withdrawal from METH (Fig. 7).

Preproenkephalin mRNA levels in striatum and accumbens of methamphetamine-treated and withdrawn rats

PPE and PPT mRNA levels were assessed in adjacent sections of brain tissue under identical conditions. Five hours after a single injection of methamphetamine (day 1), PPE mRNA levels were found elevated in all regions analyzed except the vlCPu. The highest increase was observed in the vmCPu (36% above control; Fig. 8A). Concurrent administration of MK-801 with METH neither attenuated nor potentiated the effects of METH treatment (Fig. 8A). At

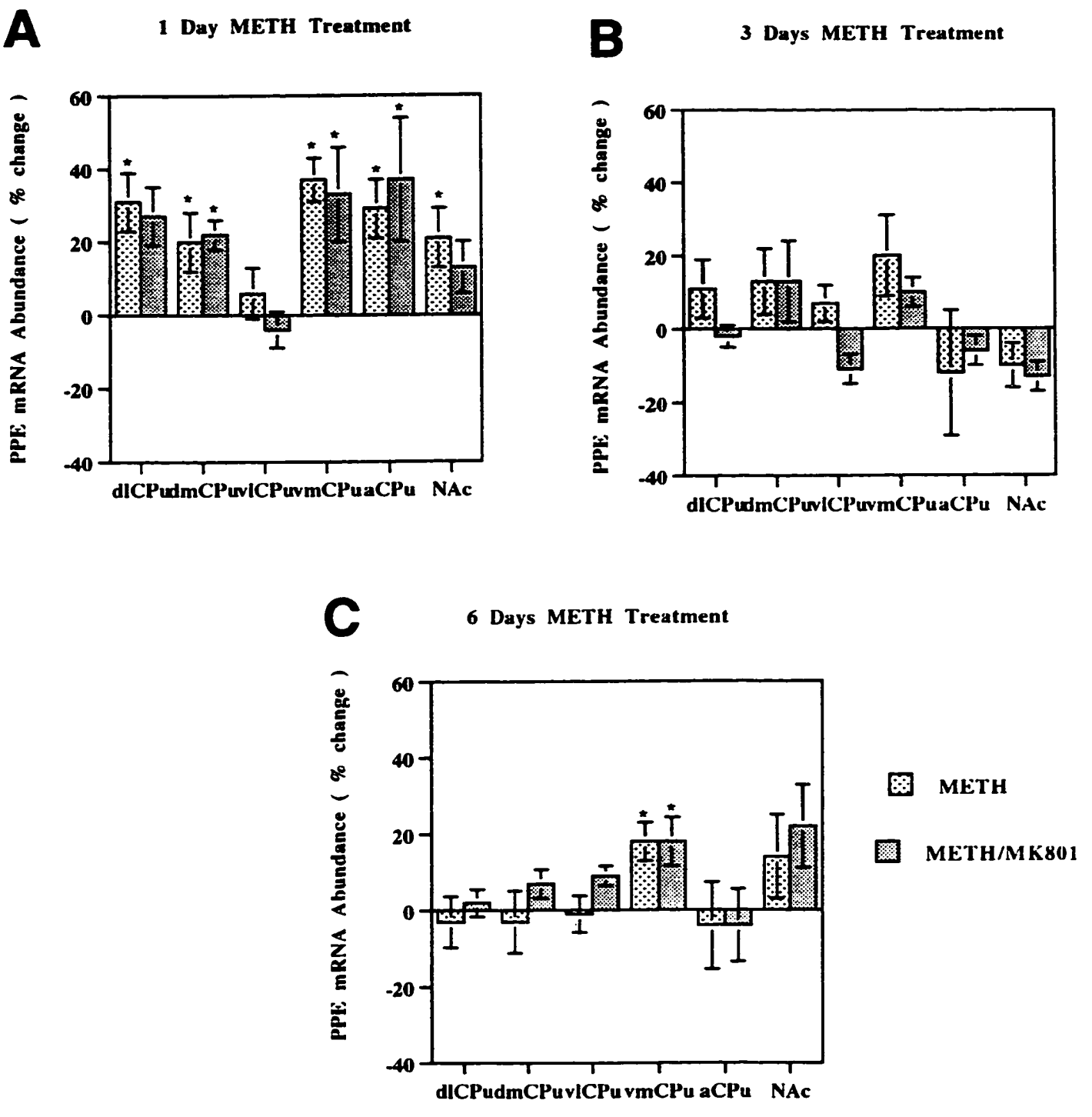


Figure 8. Time course of acute and chronic methamphetamine treatment on preproenkephalin (PPE) mRNA levels in the basal forebrain of the rat. PPE mRNA was detected by in situ hybridization histochemistry in coronal sections of brain tissue at the levels of the caudate-putamen and nucleus accumbens. PPE mRNA levels are expressed as percentage change relative to controls. PPE mRNA levels were determined from adjacent sections derived from the same set of rats (n=8 per treatment group) used for PPT mRNA (as described in legend to Fig. 6). NAc,nucleus accumbens; aCPu, anterior caudate-putamen; dlCPu, dmCPU, vlCPU and vmCPU: dorsolateral, dorsomedial, ventrolateral and ventromedial aspects of the caudate-putamen, respectively. Values over the bars represent SEM. * $p< 0.05$ (paired Student's t=test). (See Neuropharmacology, 36, 330)

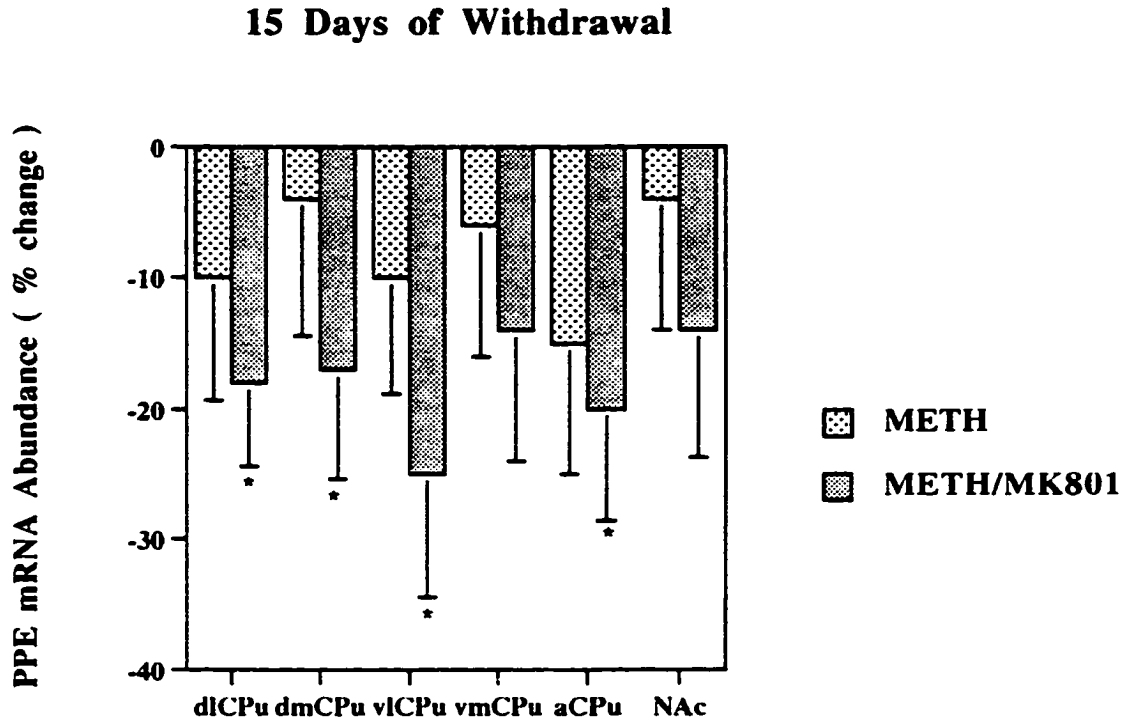


Figure 9. Assessment of preproenkephalin mRNA levels in the caudate-putamen and nucleus accumbens of the rodents withdrawn from chronic methamphetamine treatment for 15 days. PPE mRNA levels were detected in coronal sections of brain tissue by *in situ* hybridization histochemistry. Messenger RNA levels were determined with an image analysis system by estimating optical density values from X-ray autoradiograms and PPE mRNA levels are expressed as percentage change relative to controls. Rats (n=8 per treatment group) were treated with METH or METH/MK-801 (as described in legend to Fig. 7) and were sacrificed 15 days after the last injection. Positive values over the bars represent SEM values. NAc, nucleus accumbens; aCPu, anterior caudate-putamen; dlCPu, dmCPu, vlCPu and vmCPu: dorsolateral, dorsomedial, ventrolateral and ventromedial aspects of the caudate-putamen, respectively. *$p < 0.05$ (paired Student's *t*-test). (Published in Neuropharmacology (1997) 36, 331)

day 3 of treatment, the effects of acute METH on PPE mRNA levels had waned (Fig. 8B). Although the vmCPu displayed an increase of nearly 20% above control, it did approach statistical significance at the 95% confidence level due to large interanimal variability (Fig. 8B). Similarly, at day 6 of treatment PPE mRNA levels were elevated only in the vmCPu (Fig. 8C).

Withdrawal from METH for 15 days resulted in small but consistent decrease of PPE mRNA levels throughout the CPu and NAc (Fig. 9); however, these changes did not reach statistical significance. In contract to PPT mRNA, rats treated concurrently with MK-801 and METH displayed statistically significant decreases of PPE mRNA levels in dorsolateral CPu (dlCPu), dmCPu, vlCPu and aCPu (18%, 16%, 25% and 20%, respectively) at day 15 of withdrawal (Fig. 9).

DISCUSSION

Here we show that repeated injections of METH for 6 consecutive days, a paradigm that induces behavioral sensitization as well as dopamine autoreceptor subsensitivity and mesoaccumbens dopamine D1 receptor supersensitivity (Wolf and Khansa, 1991; Wolf et al., 1994), resulted in robust elevation of preprotachykinin mRNA levels in the caudate putamen and nucleus accumbens. In contrast, neostriatal PPE mRNA levels were not significantly altered by METH treatment after either 3 or 6 days of repeated injections. However, PPE mRNA levels were modestly elevated in the caudate putamen and nucleus accumbens 5 hr

after a single injection of METH. The early and transient elevation of PPE mRNA by METH may suggest that enkephalinergic projection neurons are not involved in the development of locomotor sensitization to psychomotor stimulants. The acute and transient elevation of PPE mRNA levels in the striatum has been observed and reported by another group utilizing amphetamine instead of METH (Wang and McGinty, 1995). The transient elevation of PPE mRNA by acute treatment with METH is not likely to result from psychostimulant-induced elevation of synaptic dopamine, since dopamine exerts tonic inhibitory effects on this peptidergic system (Angulo and McEwen, 1994). The early and transient increase in PPE mRNA may be mediated by intrinsic striatal mechanisms yet to be elucidated, conceivably at the level of peptidergic interactions with classical transmitters.

We assessed the effect of concurrent administration of methamphetamine and NMDA receptor antagonist MK-801, because previous studies have shown that the induction of behavioral sensitization to amphetamines requires the participation of the NMDA component of the glutamate receptor (Wolf et al., 1994). Deafferentation studies suggest that most NMDA receptor sites of the striatum are intrinsic (Greenamyre and Young, 1989) and at least some of these sites might be located on dopaminergic terminals of the striatum (Roberts and Sharif, 1978; Roberts et al., 1982). Various studies have demonstrated that NMDA receptor activation with selective agonists potentates the release of dopamine from synaptosomal preparations, slices of striatal tissue, as well as from freely moving rats (Araneda and Bustos, 1989; Kashihara et al., 1990; Krebs et al., 1991; Wang, 1991). We found that coadministration of MK-801 with METH partially inhibited

the elevation of striatal and accumbal PPT mRNA levels, except in the vmCPu where it potentiated PPT mRNA expression at day 1 of treatment (the mechanism subserving this paradoxical effect remains to elucidated). MK-801 is reported to simulate the firing rate of dopamine neurons in the mesencephalon (Zhang et al., 1992), but striatal basal dopamine release is not significantly affected by MK-801 (Morari et al., 1993). Thus, the inhibitory effect of MK-801 on METH-induced elevation of neostriatal PPT mRNA may be due to its modulatory effects on NMDA receptor-mediated actions of dopamine in the mesencephalon or to direct modulation of neostriatal neuropeptide expression at sites post-sympatic to dopamine. Chronic treatment with NMDA receptor antagonists or cortical ablation lesions support the hypothesis that glutamate activity exerts positive effects on PPE and PPT mRNA expression in the striatum of the rat brain (Somers and Beckstead, 1992; Zhang et al., 1996).

The manner in which an animal responds to a drug is determined not only by the duration of treatment but also by the time interval between exposures. Continuous (via subcutaneous pellets) or closely spaced treatments (minutes apart) with psychostimulants results in the development of tolerance or reduced responsiveness to the agent, while intermittent treatment (once daily or twice daily spaced a few hours apart) results in sensitization or reverse tolerance, i.e. increased responsiveness to inputs (Nelson and Ellison, 1978; Post, 1980). The sensitization to repetitive treatment with amphetamines can last for weeks and months after cessation of treatment (Nelson and Ellison, 1978; Paulson et al., 1991). In the present study, we found PPT mRNA levels elevated in the neostriatum 5 hr after

repetitive treatment with methamphetamine for 6 consecutive days. However, PPT mRNA levels returned to control levels at day 15 of withdrawal. It is likely that the increase in the mRNA encoding the neuropeptides substance P and neurokinin A represents a homeostatic adaptation in order to maintain higher levels of peptide utilization during drug treatment. At the organismic level, elevated activity or output of the tachykinin system via the direct striatonigral pathway may account, in part, for the enhancement of locomotor behavior associated with repetitive exposure to amphetamines. Substance P has been demonstrated to stimulate the activity of dopaminergic neurons of the substantia nigra compacta (James and Starr, 1977; Olpe and Koella, 1977). Thus, it is likely that increased levels of substance P may account in part for sensitization to methamphetamine, because this neuropeptide facilitates dopaminergic function via striatonigral-nigrostriatal reciprocal connections and might exert an amplification effect on this loop.

Repetitive treatment with methamphetamine alters some neurochemical parameters of the basal ganglia during treatment, while other interrelated parameters may be affected days or even weeks after cessation of treatment. For example, PPT mRNA levels of the neostriatum are elevated at the end of the treatment but not after 15 days of drug abstinence. In contrast, tyrosine hydroxylase mRNA levels in the ventral tegmental area are not affected after 6 days of daily injections of METH, but become elevated at day 15 of withdrawal (Zhang and Angulo, 1996). Moreover, chronic treatment with the psychomotor stimulant cocaine resulted in dopamine autoreceptor subsensitivity in the nucleus accumbens immediately after the last drug administration (Pierce et al., 1995). Neurochemical

adjustments in response to treatment to psychostimulant drugs of abuse can occur during drug administration and might represent mechanisms of tolerance to the drug. By contrast, other neurochemical changes occur much later and may be involved in the enduring aspects of sensitization to the drug.

In conclusion, progressive treatment with methamphetamine elevated preprotachykinin mRNA levels in all aspects of the caudate-putamen and accumbens in a stepwise manner between days 1 and 6 of treatment; however, PPT mRNA levels returned to control values 15 days after cessation of treatment (withdrawal). Coadministration of the NMDA receptor antagonist MK-801 attenuated the elevation of PPT mRNA observed after chronic treatment with METH for 6 consecutive days. In contrast, preproenkephalin mRNA levels were significantly affected by acute treatment with METH and decayed by day 6. The data suggest that the tachykinin system is a direct target of the actions of methamphetamine, and perhaps psychomotor stimulants in general, on the dopaminergic system of the rodent brain. Moreover, enhanced activity observed during repetitive and progressive treatment with psychostimulants. Since the effects of METH on PPT mRNA decay when the drug is withdrawn, it is likely that this peptidergic system may not subserve a neuroadaptive role sustaining enduring sensitization to amphetamines, but may play a role in the progressive augmentation of locomotor activity elicited by this class of drug.

CHAPTER IV

Extracellular Dopamine Release in Response to Acute and Challenge METH and Cocaine Treatments

INTRODUCTION

Methamphetamine and cocaine are commonly abused psychomotorstimulants that cause addiction and hyperlocomotor activity. Although methamphetamine and cocaine affect monoamine systems including dopamine, serotonin and norepinephrine, several studies indicate that the locomotor stimulating effect of amphetamine and cocaine may be associated with an increase in dopamine transmission in the axonal terminal region of dopamine neurons. Because locomotor responses to amphetamine and cocaine were prevented by concurrent treatment with dopamine receptor antagonists or destruction of dopaminergic systems (Crease and Iversen, 1975; Kelly and Iversen, 1975; Scheel-Kruger et al., 1977; Roberts et al 1980; Stewart and Vezina, 1989), most studies on psychomotorstimulants induced behavioral sensitization have focused on the dopamine systems.

Repeated injection of amphetamine and cocaine induces behavioral sensitization, which is characterized by an augmentation in locomotor activity. Behavioral sensitization has been postulated to result from enhanced dopamine release in the mesoaccumbens and nigrostriatal dopaminergic terminals in response to a challenge injection of psychostimulants. *In vitro* studies supported this hypothesis by showing enhanced dopamine release from accumbal and striatal tissue slices and synaptosomes from rats sensitized to psychomotorstimulants (Robinson and Becker, 1982; Kolta et al., 1985; Castaneda et al., 1988; Yamada et al 1988, and Kalivas and Duffy, 1988). Similarly, *in vivo* microdialysis studies demonstrated that a challenge dose of psychomotorstimulant subsequent to daily amphetamine or cocaine pretreatment produced an increase in dopamine release in both caudate-putamen and the nucleus accumbens (Robinson et al.,

1988; Akimoto et al., 1989; Kalivas and Duffy, 1990; Pettit et al., 1990). However, other studies have found that a challenge injection of amphetamine or cocaine during the early withdrawal period following repeated psychomotorstimulant injections decreased extracellullar dopamine concentration in both caudate-putamen and nucleus accumbens when compared with dopamine release induced by acute psychomotorstimulant administration (Hurd et al., 1989; Segal and Kuczenski, 1990a and 1990b; Kalivas and Duffy, 1993). Thus, whether enhanced dopamine release in the nigrostriatal and mesoaccumbal dopamine terminal regions is always associated with behavioral sensitization needs to be evaluated.

While most investigations on behavioral sensitization have focused on the terminal regions of the dopaminergic system, relatively few studies have examined the effects of systemic amphetamine or cocaine application on somatodendritic dopamine release. Dopamine cell bodies in the midbrain are the primary source of the dopaminergic input to the caudate-putamen and the nucleus accumbens. Dopamine neurons in the ventral tagmental area and the substantia nigra compacta send their axons to the nucleus accumbens and the caudate-putamen, respectively. The neuronal activity of dopamine neurons in the somatodendritic field influence the synthesis and release of dopamine in the terminal regions. Because release of dopamine in the dopaminergic terminal field was altered following methamphetamine or cocaine administration, it is important to assess dopamine efflux in the somatodendritic regions in the sensitized animals as well. Moreover, ventral tagmental area and substantia nigra are critical for the initiation of behavioral sensitization to psychostimulants, since intra-VTA and substantia nigra injection of amphetamine resulted in enhanced locomotor response to a systemic challenge injection

of psychomotorstimulants (Dougherty and Ellinwood, 1981; Kalivas and Weber, 1988; Vezina and Stewart, 1990).

To study the discrepancy reports on dopamine release in response to amphetamine or cocaine challenge injection and to find out whether enhanced dopamine release is necessary for the expression of behavioral sensitization to both drugs, the present study was designed to examine the effects of two different psychomotorstimulant, methamphetamine and cocaine, on extracellular dopamine release in both dopaminergic terminal areas: caudate-putamen and nucleus accumbens; and somatodendritic regions: ventral tagmental area and substantia nigra compacta. It is one of the major strengths of this study to contrast psychostimulant-evoked dopamine release in terminal field areas and somatodendritic areas, utilizing the same sensitization paradigm. Extracellular dopamine concentrations were measured by *in vivo* microdialysis. Experimental rats were divided into acute and challenge treatment groups. In the acute group, dopamine content was measured in drug naïve rats in response to only one dose of methamphetamine (1 mg/kg, ip) or cocaine (10 mg/kg, ip) injection. In the challenge group, release of dopamine is determined following a challenge injection after 3 days of withdrawal from 7 daily methamphetamine (1 mg/kg, ip) or cocaine treatments (10 mg/kg, ip).

METHODS

Animal housing and surgery.

Male Sprague-Dawley rats (Charles River, New York) weighing approximately 250-350g were housed in a temperature-controlled environment under a 14 : 10 h light / dark

cycle with free access to food and water. Rats were allowed to acclimate for at least 1 week after arrival in the animal facility.

Surgical Procedure

Rats (250-350g) were anaesthetized with Ketamine and Promace and placed in a stereotaxic frame (David Kopf). Holes were drilled in the skull and guide cannulas (CMA) slowly lowered into the brain over an approximate 10 min. period. Each rat was implanted with only one guide cannula. The coordinates used for the implantation of guide cannulas from bregma and the skull surface are: dCPu: A, 1.6mm; L, 2.5mm; V, 4.5mm; NAc: A, 1.6mm, L, 1.5mm, V, 7mm; SNc: P, 5mm; L, 2mm; V, 8mm; VTA: P, 5mm, L, 1mm, V, 8.5mm (Paxinos and Watson, 1986). Dental cement and 2 stainless steel screws tapped into the skull secured the cannula. The wounds were sutured. After surgery, rats were housed individually in standard rat cages and allowed 3 days of recovery prior to drug administration. Drug injections were performed in home cages.

Microdialysis and Sample Collection

On the day of measuring dopamine, rats were transferred to the dialysis testing bowl. Dialysis probes were purchased from CMA, with 2 mm of active dialysis membranes exposed at the tip. Prior to placing the probes in the rat, probe recovery was determined in vitro using a standard solution of DA. Dialysis probes were inserted through guide cannulas into the brain and were perfused at .5ul/min. with artificial cerebrospinal fluid (155.0 mM Na^+, 1.1mM Ca^{++}, 2.9 mM K^+, 132.8 mM Cl^-, .83 mM Mg^{++}, pH 7.4). Once the probes were lowered into the dCPu, NAc (Fig. 10), SNc and VTA (Fig. 11), baseline

samples (30 min each) were collected for about 180 min. After stable dopamine levels were obtained in three consecutive samples, rats received an injection of METH or cocaine depending on the treatment paradigm. Fractions were collected for 2 additional hour. Dialysis probes were removed at the end of the experiment, and brains were removed for histological verification of probe placement.

High-Performance Liquid Chromatography Analysis of Recovered Samples

Dialysis samples were collected from the dCPu, NAc, SNc and VTA over a 30 min. period. into microfuge tubes containing 2 ul of .1 M perchroric acid to inhibit dopamine oxidation and frozen at -80 °C until analyzed for dopamine. Dopamine content in the samples was measured using HPLC with electrochemical detection. Dopamine was separated using a BAS reversed-phase column and analyzed with LC-4C detector (BAS). The mobile phase consisted of 25mM NaH_2PO_4; 50 mM NaCitrate; .03mM EDTA; 10 mM Diethylamine HCl; 2.2 mM Octylsulfonic acid; 10 mM NaCl with a final pH of 3.2. 30 ml of Methanol and 22 ml of Dimethylacetamine were added into 1L of above solution. The working electrode was set at +650 V. Chromatograms were scored and converted to concentrations using standard curves with Tobochrome. In vitro probe recovery for dopamine is about 13 %. Prior to each sample run, a blank consisting of an equal volume of artificial CSF is injected into the HPLC. A standard solution of dopamine was similarly injected into the HPLC system before measuring the samples, these results were used to quantify the concentration of dopamine in the unknown samples.

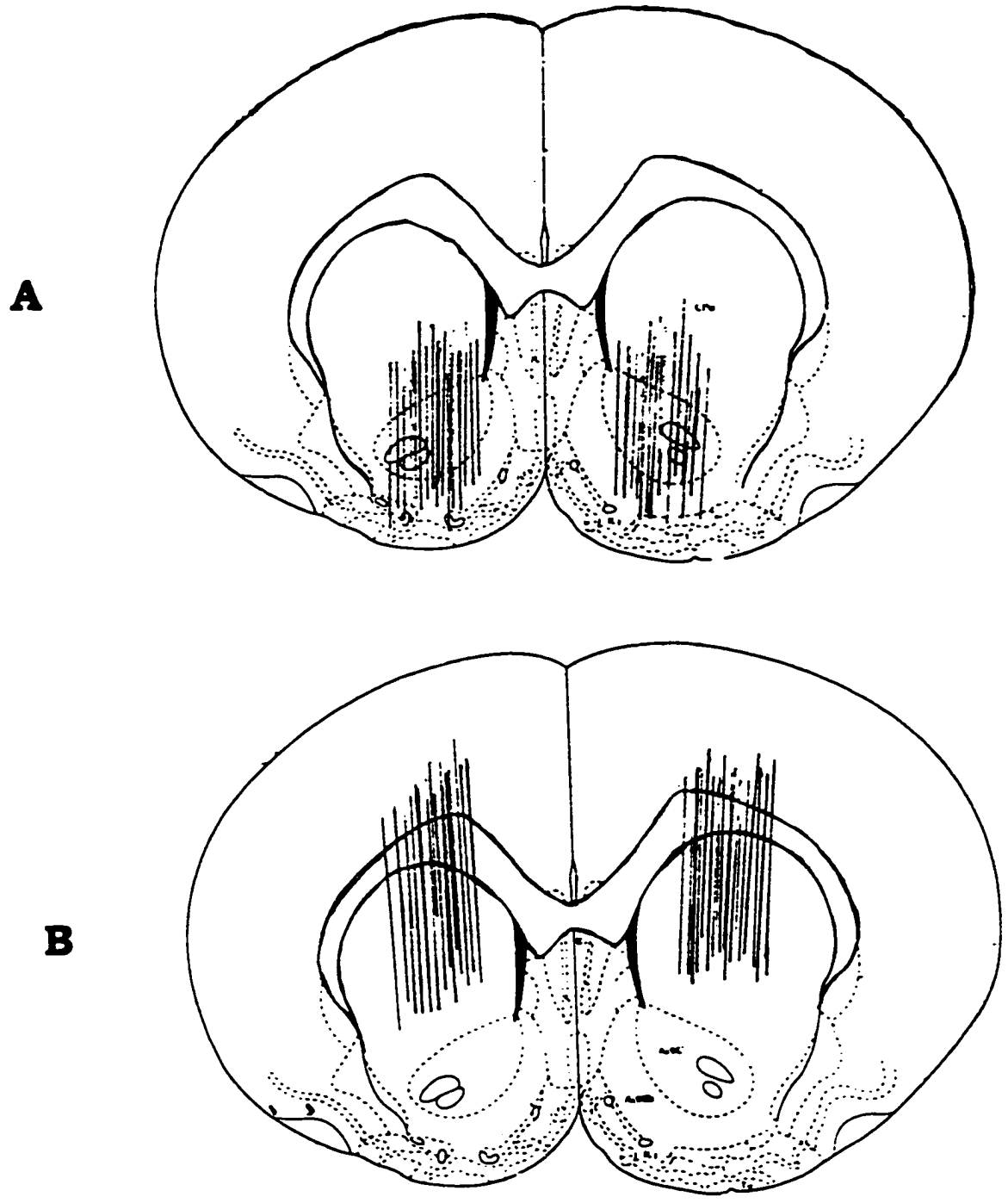


Figure 10. Location of dialysis probes in the nucleus accumbens and caudate-putamen. The vertical lines indicate the probe placement. Panel A shows the probe placement in the nucleus accumbens. Panel B shows the probe placement in the caudate-putamen. The frontal section adapted from the atlas of Paxinos and Watson (1986).

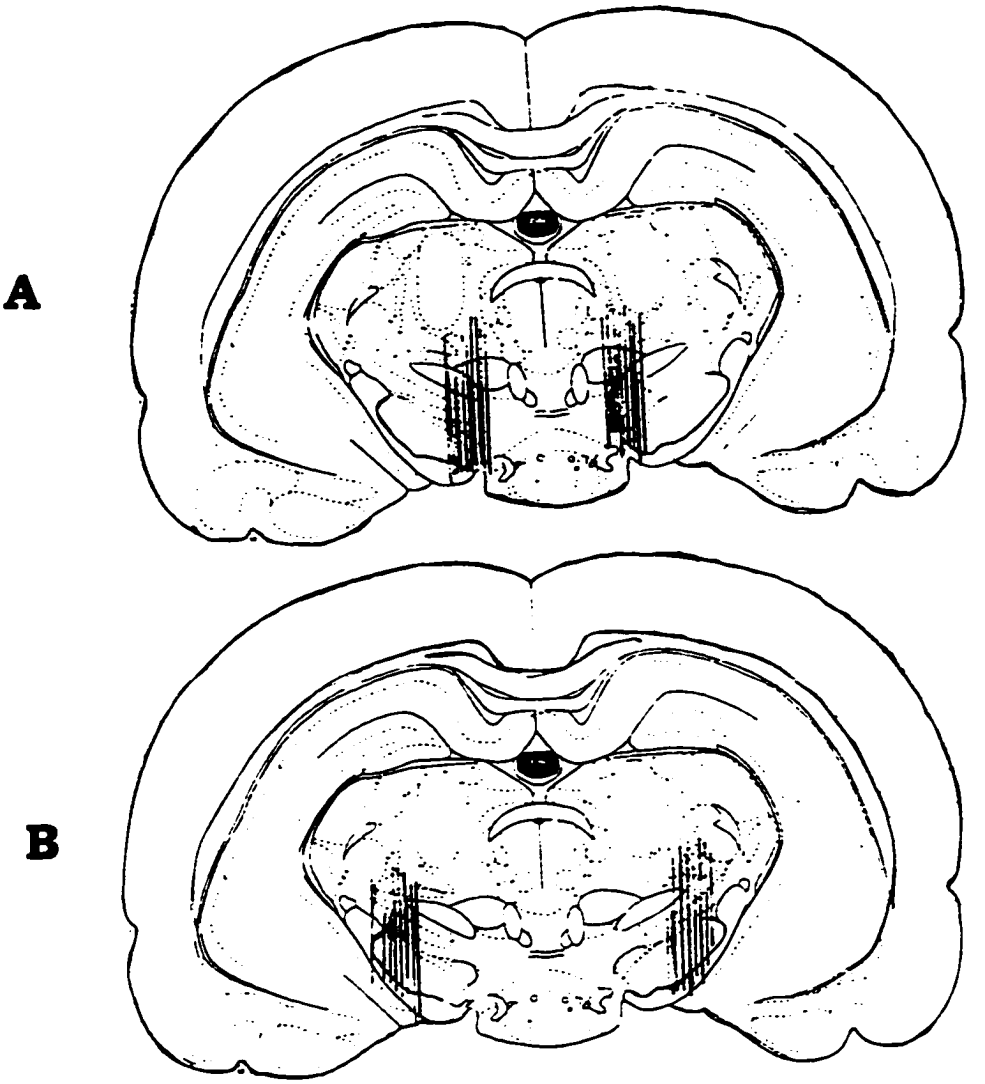


Figure 11. Location of dialysis probes in the ventromedial mesencephalon. The vertical lines refer to probe placements. Panel A shows the probe placement in the VTA. Panel B shows the probe placement in the SNc. Panel A and B are adapted from the atlas of Paxinos and Watson (1986).

Histology and data analysis

Following each experiment, rats were exposed to CO_2 for about 30 seconds before decapitation. The brains were removed from the skull and frozen at -80 °c. Probe placement was verified histologically by cutting coronal sections and visualizing the track of dialysis probe according to the atlas of Paxinos and Watson (1986) (Fig. 10 and Fig. 11). The neurochemical data were statistically analyzed using analysis of variance (ANOVA). The dopamine content was normalized to percent change and compared with the average of 3 baseline samples.

RESULTS

Effect of acute and challenge cocaine injection on dopamine release in the caudate-putamen.

Figure 12A shows that acute cocaine injection increased the level of extracellular dopamine with a maximum response (175% above baseline) at 30 min. after cocaine injection. Similarly, the cocaine challenge also elevated dopamine level about 75% above baseline. The augmentation of dopamine release in response to the cocaine challenge treatment was not as robust as that caused by acute cocaine injection. The difference of dopamine efflux between acute and challenge cocaine treatments is not statistically significant.

Effect of acute cocaine and cocaine challenge on dopamine release in the nucleus accumbens.

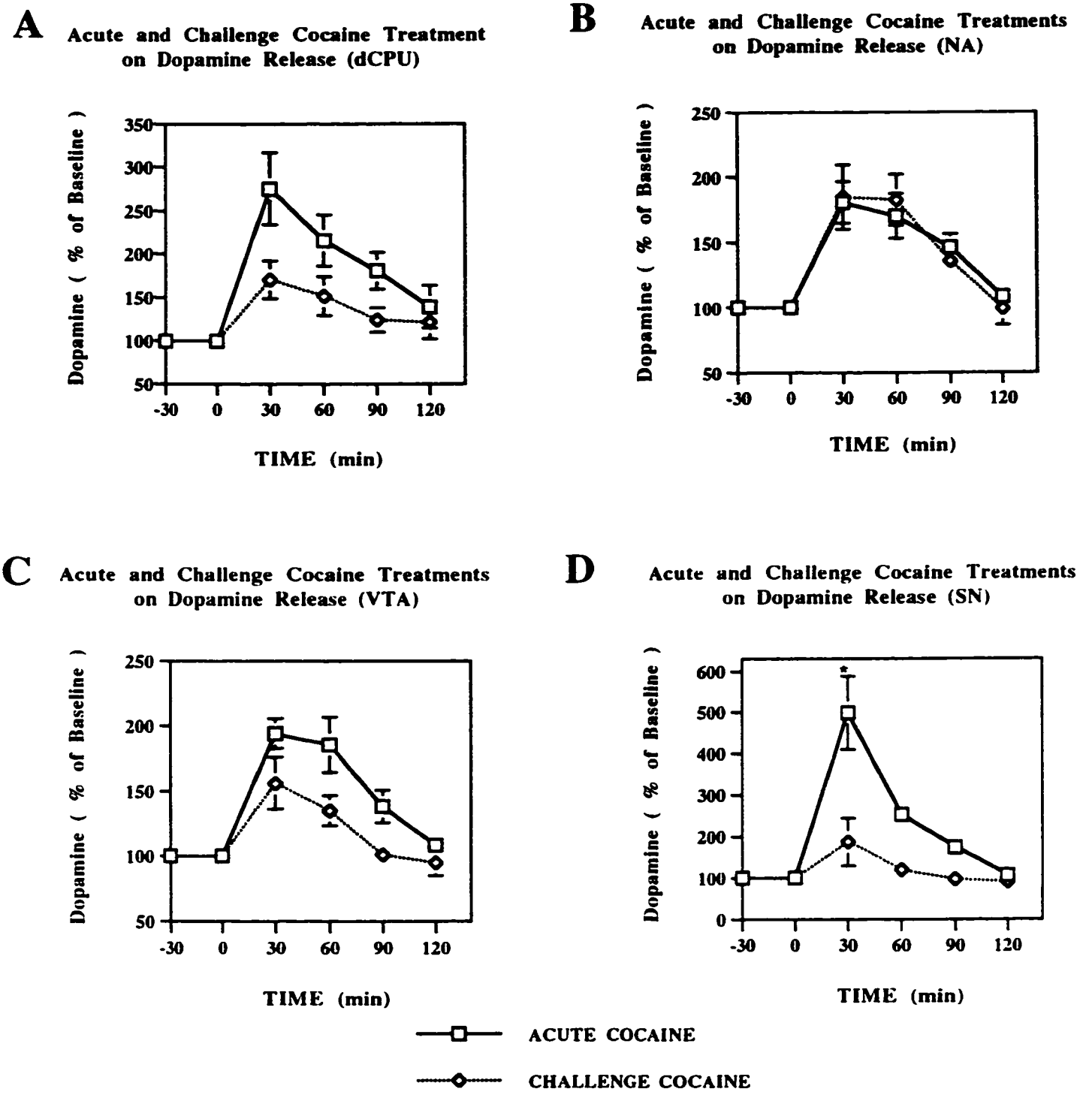


Figure 12. The effect of acute and challenge administration of cocaine on dopamine efflux in the dCPu, caudate-putamen; NAc, nucleus accumbens; VTA, ventral tegmental area; SNc, substantia nigra compacta. Extracellular dopamine in response to aucte (10 mg/kg x 1d) and challenge (10 mg/kg x 7d) cocaine injection was measured with HPLC coupled with electrochemical detector and expressed as percentage of baseline. Values above and below the lines represent the SEM. *$p < 0.05$ (paired Student's *t*-test).

As shown in Figure 12B, the challenge and acute cocaine injections produced an increase in extracellular dopamine efflux with a peak response around 30-60 min. after drug administration. There was no significant difference between the acute and challenge treatments on the release of dopamine.

Effect of acute cocaine and cocaine challenge on extracellular dopamine in the ventral tegmental area.

The effects of acute and challenge injections of cocaine on dopamine release in the VTA are shown in Figure 12C. The acute and challenge treatments evoked dopamine release immediately after the drug injections, and increase of dopamine were about 100% and 50% above baseline, respectively. The drug evoked dopamine release reached the highest levels in the first 30 min. The difference between dopamine release induced by acute and challenge injections of cocaine was not statistically significant.

Effect of acute and challenge cocaine administrations on dopamine release in the substantia nigra compacta.

Figure 12D reveals that dopamine levels in the SNc were elevated in response to the acute and challenge treatments of cocaine. The maximum responses occurred at 30 min. after cocaine injections. Acute cocaine evoked greater effect on the release of dopamine (400% above baseline) than cocaine challenge did (100% above baseline). The

difference in the extracellular dopamine concentrations in response to acute and challenge cocaine treatments was statistically significant. $P = 0.0213$ (Paired Student t test).

Effect of acute and challenge METH injections on dopamine release in the caudate-putamen.

In Figure 13A, both acute and challenge METH administrations evoked 200% increase in dopamine release. Although the extracellular dopamine concentrations reached about the same levels in response to the acute and challenge treatment, acute METH caused dopamine release with a highest response around 60 min. after drug injection. Whereas, the challenge injection elevated dopamine level with a peak response around 90 min. after the injection. The effect of METH challenge on dopamine release seemed to be longer lasting compared with the effect induced by acute METH injection. There was no significant difference in dopamine releases between the two groups.

Effects of acute and challenge METH on extracellular dopamine concentration in the nucleus accumbens.

As shown in Figure 13B, the acute and challenge injection of METH produced an augmentation in dopamine release. Naïve rats responded to acute METH injection with a 150% increase in dopamine release. Whereas the rats pretreated with METH (1 mg/kg x 7days) responded with a 400% increase in dopamine release to METH challenge injection. The difference between dopamine efflux in response to the acute and challenge injections of METH was statistically significant during the first hour after drug injection. $P = 0.0449$. (paired Student's t-test).

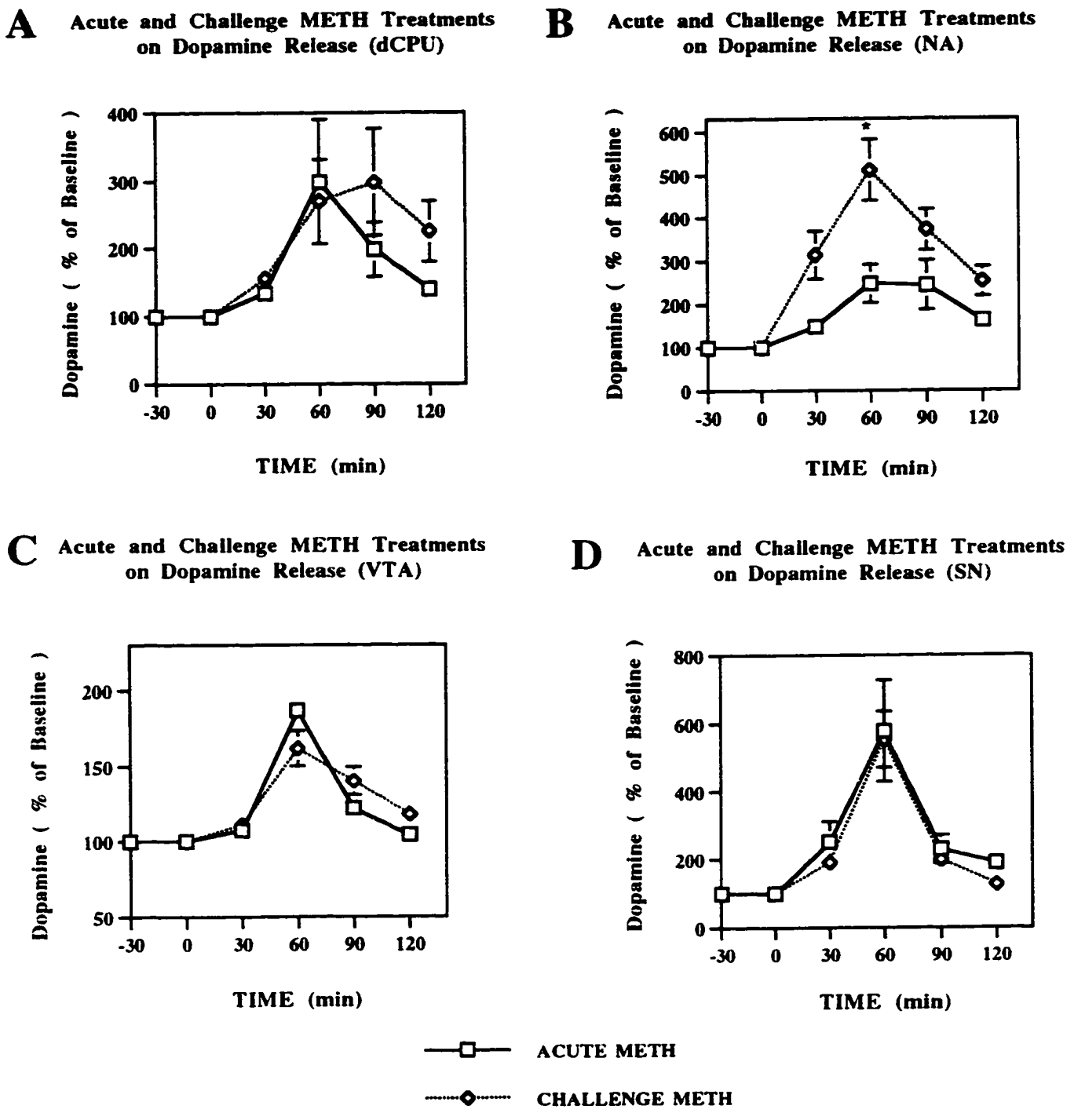


Figure 13. The effect of acute and challenge injection of METH on dopamine release in the dCPu, caudate-putamen, NAc, nucleus accumbens, VTA,ventral tegmental area and SNc, substantia nigra compacta. Dopamine is measured with HPLC coupled with electrochemical detector. Acute (1mg/kg x 1d) and challenge (1mg/kg x 7d) METH injections on dopamine efflux are expressed as percentage of basal levels. The values over and below the line represent SEM. *$p < 0.05$ (paired Student's *t*-test).

Effect of acute and challenge METH on dopamine efflux in the ventral tagmental area.

The acute and challenge injection of METH on extracellular dopamine release in the VTA is shown in Figure 13C. The highest responses in dopamine release occurred at 60 min. after drug injections. The acute METH injection increased dopamine level about 80% above baseline whereas METH challenge evoked dopamine release about 60% above the basal level. The difference between acute and challenge treatments on dopamine releases was not statistically significant.

Effect of acute and challenge METH injections on extracellular dopamine concentration in the substantia nigra compacta.

Figure 13D shows that the acute and challenge injections of METH resulted in about 500% increase in dopamine release in the SNc. The release of dopamine reached the maximum levels during the first 60 min. after METH injections. There was no significant difference in dopamine efflux between the acute and challenge groups.

DISCUSSION

The present study assessed the effect of repeated administration of either methamphetamine or cocaine on extracellular dopamine concentration in dopaminergic terminal areas of the caudate-putamen and nucleus accumbens and somatodendritic regions of the ventral tagmental area and substantia nigra compacta. The data demonstrate

that behavioral sensitization to the psychostimulant METH and cocaine could occur in the absence of enhanced extracellullar dopamine concentration.

Dopamine terminals in the caudate-putamen and nucleus accumbens are considered to be critical for the expression of behavioral sensitization to the psychomotorstimulants METH and cocaine (Kalivas, 1993), because dopaminergic transmission in these regions was altered in the sensitized rats. Numerous studies have shown that METH or cocaine pretreatment increased dopamine release following a challenge with the same drug in the caudate-putamen and nucleus accumbens (Akimoto et al., 1989; Kalivas and Duffy, 1990; Pettit et al., 1990). Similarly, in our experiments, animals pretreated with METH (1mg/kg/day x 7) showed a robust dopamine release in the nucleus accumbens and long-lasting dopamine release in the caudate-putamen in response to a challenge dose of METH. In contrast to the effect of METH on dopamine release in the dopaminergic terminal regions, pretreatment with cocaine (10mg/kg/day x 7) failed to increase the extracellular dopamine concentration in the caudate-putamen and the nucleus accumbens when challenged with the same dose of cocaine. The cocaine results are comparable with some previous reports (Hurd et al., 1989; Segal and Kuczenski, 1992b; Kalivas and Duffy, 1993a). Changes of dopamine content in the extracellular space probably depend on the time after discontinuation of daily psychostimulant treatments. Indeed, Kalivas and Duffy found that dopamine release did not increase at 24 hours but enhanced during 2 to 3 weeks of withdrawal period when challenged with a dose of cocaine after repeated daily cocaine injection (Kalivas and Duffy, 1993a). In our experiment, the challenge injection of cocaine was carried out at 4 days of withdrawal after consecutive 7

daily cocaine treatment. Our results support the time-dependent dopamine release hypothesis suggested by other investigators.

Changes in extracellular dopamine content in response to the cocaine challenge injection might result from one or all of the following alterations in the dopaminergic system. 1) The structural changes in the VTA-NAc pathway. Chronic cocaine treatment produced decrease in the levels of the 3 major neurofilament proteins in the VTA (Beitner-Johnson et al., 1992), which reduced axonal transport rates and decreased the amount of TH transported from the cell bodies to the nerve terminals in the NAc. The changes in the VTA-NAc pathway provide evidence to explain why there was not much significant difference between dopamine releases in the NAc in response to acute and cocaine challenge. Similar alternations could also occur in the nigrostriatal pathway and resulting in reduction of dopamine release in the dCPu of the sensitized animals. 2) Dopamine autoreceptor sensitivity might increase (Muscat et al., 1993). Activation of autoreceptors results in inhibition of presynaptic dopamine neurons, which leads to a decrease in elicited dopamine release. Thus, supersensitivity of D2 receptors would cause even less dopamine release. 3) The amount of dopamine in the synaptic cleft depends largely on the function of the dopamine transporter. The decrease in dopamine release in response to the cocaine challenge injection may be due to augmentation in the functional states of dopamine transporter. Indeed, enhancement of 3H-dopamine uptake in tissue slices has been observed at early withdrawal time (Missale et al., 1985; Yi and Johnson, 1990). Increase in dopamine reuptake might result in a decrease or no significant change in dopamine release measured by *in vivo* microdialysis.

Behavioral sensitization to cocaine is sustained although dopamine release in the dCPu and NAc did not increase at day 4 of withdrawal from cocaine. This disassociation between dopamine release and hyperlocomotion could occur for the following reasons: 1) The D1 dopamine receptors may be supersensitive. Several studies have found that dopamine D1 receptors are hypersensitive in sensitized rats (White, 1993). Even though the amount of dopamine released into the synapses was not increased, hyperresponse of the postsynaptic receptors may compensate for decrease in releasable dopamine. 2) Changes in other neurotransmitters. Glutamate is another important neurotransmitter involved in behavioral sensitization to psychostimulants. Studies have found that glutamate and dopamine regulate each other's release in the neostriatum (Clow and Jhamandas, 1989; Maura et al., 1988; Wang, 1991). Even though dopamine efflux is not increase significantly following cocaine challenge in the NAc, glutamate release was increased in response to the cocaine challenge in the NAc. This result will be shown in the next chapter (see Figure 14B). 3) Changes in neuropeptides in the neostriatum. For example, tachykinin mRNA levels were found to increase significantly in sensitized animals (see chapter III). As reported previously, tachykinin peptides increased dopamine release and promoted locomotor activity when injected into the VTA (Baruch et al., 1986; Pettit and Glowinski, 1986; Hokfelt et al., 1991). Thus, tachykinin peptides possibly play a role in behavioral sensitization. This is something we are pursuing in the lab right now, and the preliminary data show that sensitization is potentiated by acute administration of NK3 antagonists.

Dopamine neurons located in the ventral tagmental area and the substantia nigra compacta project to the nucleus accumbens and the caudate-putamen, respectively. The VTA is critical in the initiation of behavioral sensitization to psychomotorstimulants, since

daily injection of psychomotorstimulants into the VTA produces behavioral sensitization to a systemic psychomotorstimulant challenge (Dougherty and Ellinwood, 1981; Kalivas and Weber, 1988; Vezina and Stewart, 1990). Moreover, intra VTA injection of dopamine D1 receptor antagonists prevented behavioral sensitization induced by systemic administration of psychomotorstimulants (Stewart and Vezona, 1989). Thus, it is reasonable to predict that sensitization is accompanied by an increase in dopamine release in the VTA and the SNc. Indeed, Kalivas and Duffy showed that somatodendritic dopamine release was augmented in response to a challenge treatment at 24 hours after discontinuation of repeated injection of cocaine (Kalivas and Duffy, 1993b). Surprisingly, our experiments demonstrated the opposite: acute treatment of cocaine increased dopamine release more than that induced by a challenge injection to rats pretreated with cocaine in both the VTA and SNc. Similarly, METH challenge failed to increase dopamine release in the VTA and SNc compared with that induced by acute METH.

There are two possibilities to explain the difference between our data and the previous report. 1). The doses and treatment paradigms applied in each experiment are different. The previous study used a paradigm (15mg/kg, ip x 1d followed by 30mg/kg, ip x 5d), which is larger in dosage compared with ours. And the challenge injection was done 1 day after discontinuation of repeated cocaine treatment. In our experiment, the challenge cocaine was injected 4 days after discontinuing daily cocaine treatment (10 mg/kg, ip x 7d). 2) The increase in dopamine release in response to cocaine challenge reported by Kalivas and Duffy (Kalivas and Duffy, 1993b) was hypothesized to result from subsensitivity of dopamine D2 autoreceptors in the VTA and SNc. However, subsensitivity of dopamine D2 autoreceptors in the VTA generally lasts for 1 to 4 days after discontinuation of cocaine

injections (Henry et al., 1989, White et al., 1995). In the same studies performed by Kalivas and Duffy, cocaine challenge failed to enhance dopamine release in the somatodendritic regions 14 days after discontinuing repeated cocaine treatment (Kalivas and Duffy, 1993b). Thus, decrease in dopamine release or lack of change in dopamine release in response to psychostimulant challenge found in our studies may be due to the fact that the dopamine autoreceptor become supersensitive (Gawin and Ellinwood, 1988, Zhang et al., 1992).

In conclusion, our study found that behavioral sensitization to METH is associated with an increase in dopamine release in the NAc and long-lasting dopamine release in dCPu. In contrast, behavioral sensitization to cocaine occurred in the absence of augmentation of dopamine release in the NAc and the dCPu. In the somatodendritic area, dopamine levels did not change in response to METH challenge and was decreased in response to cocaine challenge. These findings suggest that behavioral sensitization could sustain without an increase in the neurotransmitter dopamine levels in the axonal terminal and the somatodendritic regions of the midbrain dopamine systems. Thus, an increase in locomotor activity in response to a challenge injection of psychomotorstimulants may result from changes in other aspects of the dopamine system or other neurotransmitter systems of the basal ganglia.

CHAPTER V

Glutamate Efflux in the Basal Ganglia in Response to Acute and Challenge Cocaine and METH Injections

INTRODUCTION

Although investigations on behavioral sensitization induced by psychomotorstimulants have focused on mesencepholic dopaminergic systems, converging evidence suggests that glutamatergic neurotransmission also plays an important role in behavioral sensitization. The involvement of excitatory amino acids in behavioral sensitization is indicated by the following observations: 1). Coadministration of NMDA and non-NMDA receptor antagonists with psychomotorstimulants blocks the initiation and expression of behavioral sensitization (Karler et al., 1989; 1991; Wolf and Khansa, 1991; Stewart and Druhan, 1993). 2). Sensitization-related changes in the mesoaccumbens DA system such as D1 receptor supersensitivity and autoreceptor subsensitivity are prevented by coadministration of the NMDA receptor antagonist MK-801 (White et al., 1993; Wolf et al., 1994). 3). Neurons in the VTA show hypersensitivity to glutamate application, whereas neurons in the NAc are hyposensitive in the sensitized animals (White et al., 1993). Thus, taken together, glutamatergic neurotransmission is involved in behavioral sensitization to psychomotorstimulants.

The neostriatum receives massive glutamatergic innervations from the cortex and thalamus (Webster, 1961; Kitai, 1981; Beckstead, 1984; Gerfen, 1984). These efferents provide the major excitatory input on striatal neurons, since stimulation of the cortex and thalamus results in EPSP in the striatal neurons (Wilson et al., 1982, 1983). Because the direct and indirect striatonigral pathways, emanating from the neostriatum, are involved in locomotion control, glutamate release in the neostriatum may play an important role in behavioral sensitization. Moreover, the corticothalamostriatal inputs not only excite

neurons within the neostriatum, but also regulate dopamine release from the dopaminergic terminals. Indeed, studies have shown that glutamate can increase the release of dopamine in striatal slices, synaptosomes or dialysates through its actions at NMDA, non-NMDA and metabotropic glutamate receptors (Clow and Jhamandas, 1989; Sacaan et al., 1992; Shimizu et al., 1990; Wang, 1991).

Numerous studies on behavioral sensitization have focused on the VTA because repeated microinjection of amphetamine into the VTA produces behavioral sensitization to systemic challenge of amphetamine or cocaine (Kalivas and Weber, 1988; Vezina and Stewart, 1990; Hooks et al, 1992). Moreover, blockade of NMDA receptors in the VTA prevents the initiation of behavioral sensitization (Kalivas and Alesdater, 1993). Dopamine neurons in the VTA receive excitatory amino acid afferents from the prefrontal cortex (Carter, 1982; Christie et al., 1989). Stimulation of these projections evokes burst firing patterns in VTA dopamine neurons (Gariano and Groves, 1988), through excitation of the NMDA receptors (Overton and Clark, 1992; Johnson et al., 1992). Increase in firing rate of dopamine neurons may lead to an elevation in axonal dopamine release (Gonon, 1988; Suaud-Chagny et al., 1992).

Behavioral sensitization induced by repeated administration of psychostimulants may be associated with gradual elevation of glutamate release in the VTA and SNc. This view is supported by the observation that systemic administration of cocaine and injection of D1 receptor agonist into the VTA result in an increase in the extracellular glutamate (Kalivas and Duffy, 1995). In addition, changes in the efflux of glutamate may eventually lead to alteration of the glutamate receptors, thus changing the firing rate of dopaminergic neurons in the midbrain and resulting in changes in dopamine release.

The present experiment is designed to examine extracellular glutamate in the basal ganglia. Extracellular glutamate is measured in animals treated with either acute or challenge cocaine or METH. Changes in glutamate release are compared with changes in dopamine release in animals under the same drug treatment paradigm, through which interaction between glutamate and dopamine related to behavioral sensitization can be studied.

METHODS

Animals and drug treatment

Male Sprague-Dawley rats (Charles River) weighing 250-300 g were housed 2 per cage in the animal facility. The room was maintained at a constant temperature and humidity with a 12-h light : dark cycle. Rats were allowed to acclimate for at least 1 week to the colony room before surgical implantation of guide cannulas. Rats were housed alone after the surgery and allowed to recover from the surgery for 3 days. Some rats received no further treatment until used for the microdialysis. Others were injected with methamphetamine (1 mg/kg x 7days) or cocaine (10 mg/kg x 7days) in their home cages. Injections were performed in the morning. Microdialysis experiments were carried out 3 days after the last injection for assessment of sensitized responses.

In vivo microdialysis

Guide cannulas were stereotaxically implanted over the target regions of animals were under Ketamine and Promace anesthesia. Stereotaxic coordinates from the bregma,

the midline and surface of the skull were as follows (Paxinos and Watson, 1986): NAc: A 1.5, L 1.5, V 7; dCPu: A 1.5, L 1.5, V 4.5; SN: P 5.2, L 2.0, V 8; VTA: P 5.0, L .5, V 8.5. On the day of experiment, rats were transported to the dialysis room, placed in the testing bowl. After at least 1 hr habituation, probes were inserted. Artificial CSF was constantly perfused through the microdialysis probe, and the perfusion rate was 0.5ul/min. Samples were collected every 30 min for 3 hours to establish a stable baseline. Challenge injection was performed about 3 to 3.5 h after the probe insertion. The artificial CSF contains 155.0 mM Na^{+}, 1.1 mM Ca^{2+}, 2.9 mM K^{+}, 132.8 mM Cl^{-}, .83 mM Mg^{2+} pH 7.4. Probes were purchased from CMA (CMA/ 10 microdialysis probes; CMA Microdialysis, Stockholm, Sweden) with a 2 -mm membrane length for NAc, dCPu, SNc and VTA. All probes were tested for in vitro recovery on the day before use. Following each experiment, rats were sacrificed. Probe placement was examined in sections stained with cresyl violet. Only data from rats with verified probe placement were included in the analysis.

HPLC

After collection, dialysates were frozen at -80°C before analysis. Precolumn derivatization with o-phthalaldehyde and B-mercaptoethanol was performed manually. While awaiting derivatization and injection, samples were maintained at 4°C. Sample and reagent were allowed to react for 2 min. Then, a portion of the mixture was injected into the HPLC column (BAS). Glutamate was detected using an electrochemical detector. Glutamate levels were quantified based on peak area by comparison with standards.

Data Analysis

Data were expressed as percent of baseline and statistically analyzed. Treatment groups were compared using ANOVA. Basal amino acid levels for each rat were defined as the mean of three values obtained immediately before drug injection. Significance was at the 95% confidence level.

RESULTS

Effect of acute and challenge cocaine on glutamate release in the caudate-putamen

Figure 14A shows that acute injection of cocaine (10mg/kg) resulted in a decrease in glutamate efflux in the dCPu during the first 30min. after drug administration. Glutamate level returned to baseline about 60 min. after cocaine injection. In contrast, a challenge injection of cocaine induced about 38% increase in glutamate efflux during the first 30 min. in the sensitized animals. The difference in glutamate levels between acute and challenge groups during the first 30 min. after drug injections was statistically significant. $P = 0.029$ (paired Student's *t*-test).

Effect of acute and challenge cocaine on glutamate efflux in the nucleus accumbens.

Figure 14B reveals systemic injection of a single injection of cocaine caused a gradual, small increase in glutamate level in the NAc. In response to a challenge injection 3 days after 7 daily administrations of cocaine, the level of glutamate was about 3 fold higher compared with the effect caused by acute injection. Release of glutamate reached the highest level in the first 30 min. after the injection under both

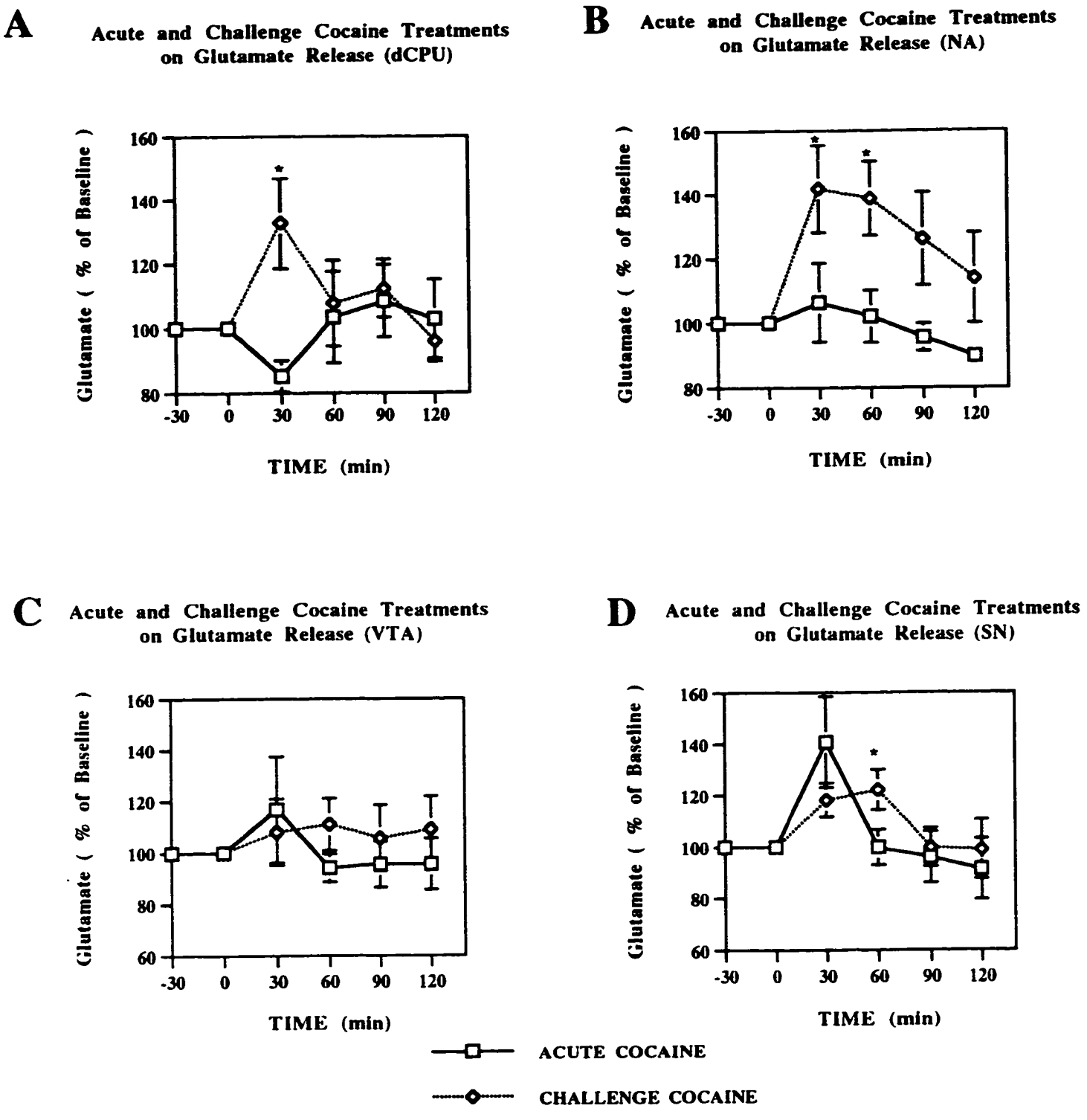


Figure 14. The effect of acute and challenge cocaine injections on glutamate efflux in the dCPu: caudate- putamen, NAc: nucleus accumbens, VTA: ventral tegmental area and SNc: substantia nigra compacta. Glutamate was collected with *in vivo* microdialysis and measured with HPLC coupled with electrochemical detector. Glutamate concentration is expressed as percentage of basal levels. Values above and below the lines represent the SEM. $*p < 0.05$ (paired Student's *t*-test).

situations. There was a significant difference in glutamate level between the acute and chronic cocaine groups during the first hour after drug injection. $P = 0.005$ (paired Student's *t*-test).

Effect of acute and challenge cocaine on glutamate release in the VTA

Figure 14C shows that a single dose of cocaine evoked glutamate release about 18% above the baseline in the VTA immediately after the injection. The concentration of glutamate then declined to the baseline. However, a cocaine challenge injection caused a gradual and small increase of glutamate release (about 10%). There was no significant difference in glutamate release between acute and challenge cocaine administrations.

Effect of acute and challenge cocaine on glutamate release in the SNc

Effect of cocaine on glutamate release in the SNc is shown in Figure 14D. An acute injection of cocaine immediately elevated extracellular glutamate level about 40% above the baseline during the first 30 min. Glutamate concentration returned back to basal levels by 60 min. after the injection. Similarly, in sensitized animal, a challenge cocaine injection also enhanced glutamate release; however, the increase was more gradual and lower than (20%) that induced by the acute cocaine injection. The alteration in glutamate release in response to acute and challenge cocaine treatments was significantly different at 60 min after drug injections. $P = 0.0325$ (paired Student's *t*-test).

Effect of acute and challenge METH on glutamate release in the caudate-putamen

As shown in Figure 15A, an acute METH injection did not change the extracellular glutamate concentration very much. Glutamate concentration was around the baseline throughout the 2 hours period after drug injection. However, a METH challenge injection increased glutamate efflux about 10% above the basal level during the first hour, then glutamate level gradually returned to baseline. Between the acute and chronic METH treatment groups, there was a significant difference in glutamate level at 60 min. after drug injection. $P = 0.0181$ (paired Student's *t*-test).

Effect of acute and challenge METH on glutamate release in the nucleus accumbens.

Figure 15B reveals that acute and systemic administration of METH resulted in an increase in glutamate efflux (10-20%). Glutamate returned to basal levels within 2 hours after drug administration. A challenge injection of METH did not evoke changes in the extracellular glutamate concentration. The alteration in glutamate level in response to acute and challenge METH treatments was significantly different at 90 min. after drug administrations. $P = 0.0201$ (paired Student's *t*-test).

Effect of acute and chronic METH on glutamate release in the VTA

In Figure 15C, acute administration of METH induced a gradual decrease in glutamate efflux in the VTA (15%), which was significantly below the baseline. By the end of the experiment, glutamate level ascended to the basal level. In contrast, a METH challenge injection seemed to elevate extracellular glutamate level, although the

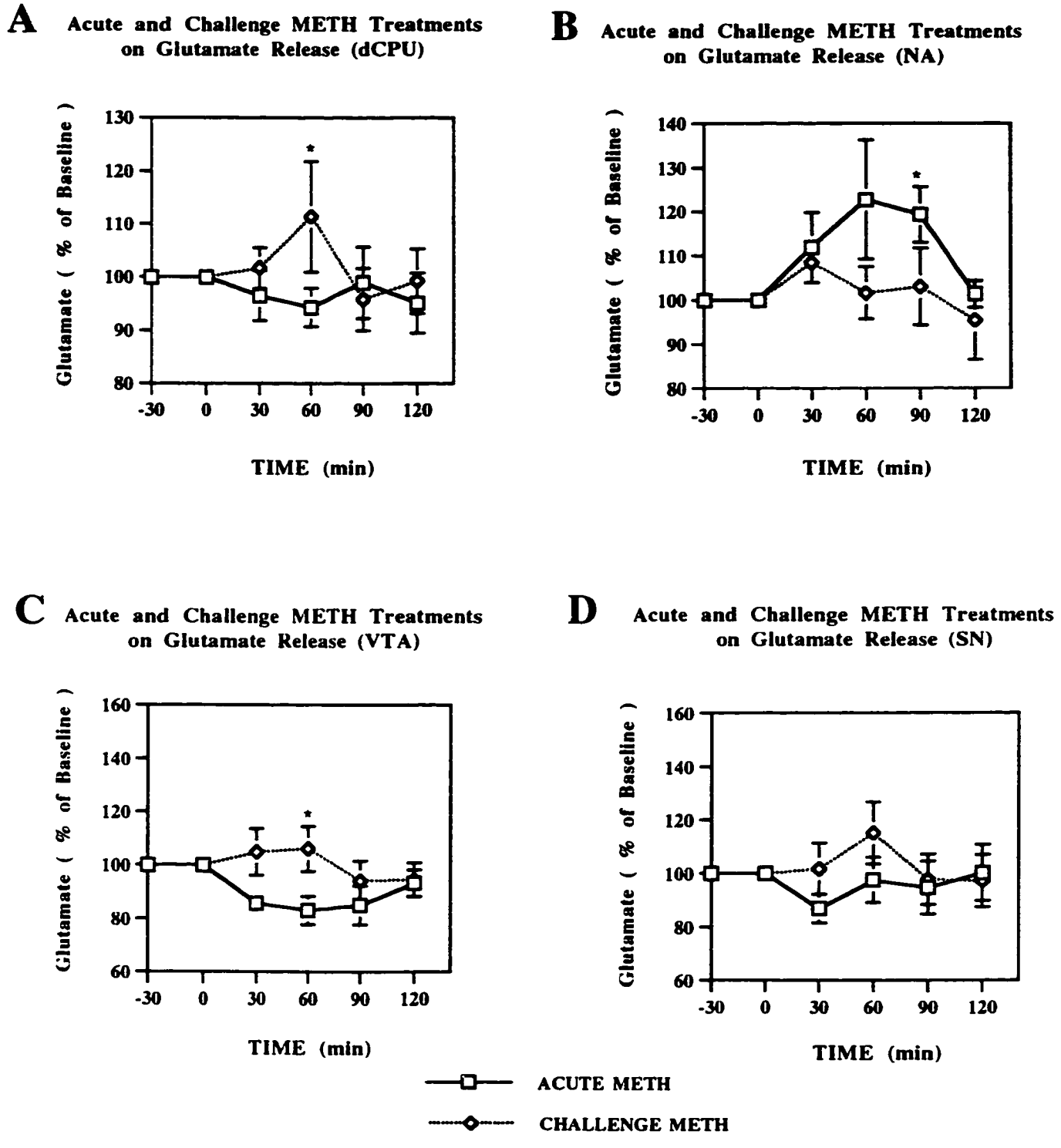


Figure 15. Effect of acute and challenge injection of METH on glutamate efflux in the dCPu, caudate-putamen; NAc, nucleus accumbens; VTA, ventral tegmental area; and SNc, substantia nigra compacta. Glutamate releases in response to acute (1 mg/kg x 1d) and challenge (1 mg/kg x 7d) METH injections are expressed as percentage of baseline. Values above and below the lines represent the SEM. $*p < 0.05$ (paired Student's *t*-test).

increase was not robust (<10%). The change in glutamate level in response to acute and chronic METH treatments was statistically significant at 60 min after METH injections. $P = 0.0343$ (paired Student's *t*-test).

Effect of acute and challenge METH on glutamate release in the SNc

Effect of METH on glutamate release in the SNc is shown on Figure 14D. Acute administration of METH decreased glutamate release during the first 30 min. after the METH injection. The extracellular glutamate concentration gradually ascended to the baseline. However, a challenge injection in rats that received repeated METH treatment for 7 days resulted in an augmentation in glutamate release (15%), especially 60 min after the drug injection. There was no significant difference in glutamate efflux between acute and challenge groups at the 95% confidence level, it is interesting to note that METH treatments in both VTA and SNc display similar pattern in glutamate release in sensitized rodents.

DISCUSSION

The present study assessed the effect of acute and challenge administration of the psychomotorstimulants methamphetamine and cocaine on extracellular glutamate release in the neostriatum, the primary structure of the basal ganglia, and the ventral mecencephalon, where dopamine cell bodies are located for mesolimbic and nigrostriatal projection to the forebrain. Our data demonstrates that extracellular

glutamate levels change in animals sensitized by repeated psychomotorstimulant administration.

Neostriatum

The neostriatum is innervated by massive glutamatergic projections from the cortex and thalamus (Fonnum et al, 1981; Reubi et al, 1979; Spencer, 1976). The corticothalamostriatal terminals form asymmetrical synapses on the spine heads of the striatal spiny neurons (Koetter, 1994). In addition, the spiny neurons are also the main target of dopaminergic innervation from the midbrain. Dopaminergic axons form symmetrical synapses on the neck or shaft of spines on the spiny neurons in the striatum (Smith and Bolam 1990). Thus, this anatomical arrangement allows dopaminergic synapses to regulate the excitatory corticothalamic inputs (Smith and Bolam, 1990). The close anatomical location, between glutamatergic and dopaminergic synapses on the spine of the striatal medium spiny neurons, provides an opportunity for the cross communication between these synapses. This allows interaction between glutamate and dopamine transmitters to occur in the striatum.

Previous research has indicated that excitatory amino acids are important in the development of behavioral sensitization to psychomotorstimulants, because sensitization is prevented by coadministration of NMDA receptor antagonists with psychostimulants. The present study shows that a psychomotorstimulant challenge injection during the early withdrawal period, following repeated psychomotorstimulant administrations (7 days), increased glutamate release in the neostriatum compared with glutamate efflux caused by acute drug treatment. The augmentation in striatal

glutamate efflux is possibly due to increase in corticothalamostriatal pathway activity. However, change of glutamate efflux in the nucleus accumbens in response to acute and challenge METH treatment are evidently more complicated. In contrast to the effect of psychomotorstimulant on glutamate release in other regions of the basal ganglia, acute injection of METH induced more glutamate release in the nucleus accumbens than that produced by METH challenge. Consequently, we have to look for alternate mechanisms to explain why sensitized animals release less glutamate in response to a METH challenge injection. Interestingly, dopamine level in the nucleus accumbens, in response to a METH challenge injection, is significantly higher than dopamine level induced by acute METH injection. Thus, increase in striatal extracellular dopamine may act on D2 dopamine receptors located in the corticostriatal terminals and inhibit glutamate release from these afferents. This finding fits well with the hypothesis that dopamine and glutamate regulate each other's release in the neostriatum.

Whether or not an increase in striatal glutamate transmission stimulates motor activity is controversial. Carlsson and Carlsson reported that MK-801, a non-competitive NMDA receptor antagonist, caused pronounced locomotor stimulation in mice depleted of monoamines (Carlsson and Carlsson, 1989). Moreover, Biggs and Star found that release of glutamate was increased in EPN in rats treated with reserpine (Biggs and Star, 1997), suggesting an increase in EAA activity in dopamine depleted animals. However, here we show that glutamate release is elevated in the striatum in sensitized rodents. Thus, glutamate may regulate locomotor activity differently depending on the status of dopamine and other neurotransmitters in the neostriatum.

Mesencephalon

Dopamine neurons in the VTA and SNc receive excitatory amino acid afferents from the prefrontal cortex and the subthalamus (Carter, 1982; Christie et al., 1989). Stimulation of these projections evokes burst firing patterns in VTA dopamine neurons (Gariano and Groves, 1988). Excitation of the NMDA receptors is responsible for the burst firing of DA neurons in the VTA and SNc (Overton and Clark, 1992; Johnson et al., 1992), leading to an elevation in axonal dopamine release (Gonon, 1988; Suaud-Chagny et al., 1992). Dopamine may activate D1 receptors located in the afferent terminals and regulate release of other neurotransmitters. For example, activation of D1 receptors may facilitate GABA release from the striatonigral terminals (Starr, 1987). Moreover, stimulation of D1 receptors could also modulate glutamate release, because the D1 antagonists prevent glutamate release induced by D1 agonist (Abarca et al., 1995). This indicates the glutamatergic afferents in the VTA and SNc could also express D1 receptors. Thus, dopamine and glutamate in the midbrain may regulate each other's release, and both neurotransmitters play important roles in the regulation of motor activity. Behavioral studies show that administration of AMPA/kainate agonists into the VTA stimulates locomotor activity (Kalivas et al., 1989; Hooks and Kalivas, 1994). This increase in locomotor activity is not observed if the animals are pretreated systemically with a dopaminergic antagonist, suggesting dopamine transmission mediates the locomotor activity induced by AMPA/kainate agonists in the VTA. It also supports the hypothesis that glutamate and dopamine in the VTA are interregulated.

Behavioral sensitization is believed to be initiated in the VTA because intra-VTA administration of MK-801 blocked behavioral sensitization (Kalivas and Alesdatter, 1993), suggesting that activation of glutamate receptors in the VTA is necessary for the development of behavioral sensitization. Thus, it is reasonable to hypothesize that locomotor sensitization to psychomotorstimulants is associated with an increase in glutamate efflux in the ventral midbrain. Consistent with this hypothesis, our studies found that challenge injections of either cocaine or METH tend to increase glutamate release in both the VTA and the SNc, even though the augmentation is not significant in some of the regions examined. However, supersensitivity of the VTA glutamate receptors in dopamine neurons was detected in sensitized animals by other investigators (White et al., 1995). Therefore, stimulant-induced glutamate efflux may enhance the responsiveness of dopamine neurons to the excitatory inputs, resulting in an increase in the firing rate of dopamine neurons and augmentation of dopamine release.

In the ventral midbrain, the effect of D1 receptor stimulation on glutamate release is biphasic, since both the D1 dopamine receptor agonists and antagonists were shown to induce glutamate release in the SN (Abarca et al., 1995). Here, acute systemic injection of the indirect dopamine agonist cocaine enhances glutamate release in the VTA and SNc. In contrast, acute METH injection does not increase, but rather decreases glutamate efflux in the same areas. The dosage of cocaine and METH used in this experiment may release different amount of dopamine based on their pharmacological effect on dopamine neurotransmission. Thus, either induction or

inhibition of glutamate release by dopamine may depend on the amount of dopamine in the synaptic clefts. This possibility needs to be clarified in the future.

In conclusion, in keeping with current proposal that behavioral sensitization is associated with glutamate hyperactivity in the basal ganglia, we have provided evidence that the striatal extracellular glutamate efflux in response to challenge psychomotorstimulants injections is increased. Moreover, the extracellular glutamate concentration in the VTA and SNc, where the dopamine cell bodies are located, is also elevated following cocaine or METH challenge. They presumably stimulate more dopamine release, which in turn increases locomotor activity. However, the neuronal origins of glutamate release, and the paradoxical lack of change in glutamate concentration in the NAc, in response to challenge METH treatments remain to be clarified.

CHAPTER VI

General Discussion

Behavioral sensitization to psychomotorstimulants METH and cocaine seems to be related to the alterations in several neuronal systems in the basal ganglia of the rat brain. The primary structure of the basal ganglia is the neostriatum. Two pathways originating from the neostriatum are associated with regulation of locomotor activity: the direct striatal and the indirect striatal pathways. Each pathway contains a variety of neurotransmitters and neuropeptides. How changes in these neurotransmitter and neuropeptide systems under psychomotorstimulant treatment would affect locomotor activity will be discussed in this chapter.

Factors Affecting the Balance between Direct and Indirect Pathways

Several neurotransmitters and neuropeptides participate in both the direct and indirect striatal pathways. Changes in the levels of these neurotransmitters and neuropeptides would result in an imbalance between the two pathways, which affects locomotor activities.

First of all, an increase in the tachykinin peptides facilitates dopamine release in the neostriatum. Tachykinin peptides are present in the direct striatonigral pathway innervating the substantia nigra reticulata. The increase in the PPT mRNA level presumably results in an increase in tachykinin peptide release. The latter would lead to more dopamine release in the terminal regions, enhance locomotor activity and potentiate the AMPH-induced locomotor stimulating effect (Baruch et al., 1986; Pettit and Glowinski, 1986; Hokfelt et al., 1991). Thus, an increase in tachykinin peptides may excite the direct striatonigral pathway. However, an increase in tachykinin

peptides also stimulates the cholinergic interneurons and increase acetycholine release in the neostriatum (Anderson et al., 1993). Acetylcholine exerts an excitatory effect on the enkephalinergic neurons forming the indirect pathway. Thus, whether or not augmentation in tachykinin peptide produces an increase in locomotion depends on the balance between the two effects mentioned above. Here we show that behavioral sensitization is accompanied with an increase in PPT mRNA expression in the neostriatum. Thus, the overall effect of increase in PPT mRNA seems to increase locomotor activity, presumably playing an important role in the expression of behavioral sensitization.

Second, dynorphin is an opioid peptide coexisting with tachykinin peptides in the direct striatonigral pathway. Previous studies showed that dynorphin exerts inhibitory presynaptic influences over glutamate and GABA release (Angulo and McEwen, 1994) in the direct pathway. Thus, dynorphin could regulate the neuronal activity in the SNr, eventually affecting locomotor activity.

Third, another opioid peptide, enkephalin, is expressed by neurons of the indirect striatonigral pathway. Enkephalin peptide expression is inhibited by nigrostriatal and mesolimbic dopamine systems (Gerfen, 1992). Previous studies found that enkephalin inhibits the further release of GABA from the same neurons (Angulo and McEwen, 1994). Our data showed that PPE mRNA was increased in response to an acute METH injection. Increase in PPE mRNA may result in an increase in the release of peptide enkephalin. The increase in enkephalin disinhibits GABA neurons of the GP, so the latter inhibits the STN, resulting in less stimulation of the GAGB neurons of the SNr. The thalamus in turn excites the motor cortex, leading to an

increase in locomotor activity. PPE mRNA level increased immediately after a single injection of METH and returned to control value after 3 and 6 days of METH injections. Thus, it is likely that enkephalin is involved in the acute locomotor stimulating effect induced by METH, but not in the progressive increase in locomotor activity in the sensitized animals. It is also possible the enkephalin peptides initiate changes within the basal ganglia that support the development of behavioral sensitization.

Fourth, the projection neurons form the direct and indirect striatonigral pathways utilize GABA as their neurotransmitter. GABA released from the direct pathway inhibits neurons in the SNr and increases locomotor activity. GABA released from the indirect pathway inhibits neurons in the GP and decreases motor activity. Thus, any factors affecting GABA release would imbalance the direct and indirect pathways. It will be interesting to see how METH and cocaine administration affects the GABA neurotransmitter release from these pathways.

Dopaminergic Terminals in the Neostriatum are from One Brain Area while Glutamatergic Terminals are from Four Different Brain Areas.

The dopaminergic input in the neostriatum is derived from the VTA and the SNc of the midbrain. Increase in dopamine release does not necessarily indicate that the neuronal activity of dopaminergic neurons is enhanced, because the firing rates of dopamine neurons were decreased following cocaine or methamphetamine administration. Rather, an increase in dopamine release in response to

psychomotorstimulant cocaine and METH injections may suggest that more dopamine accumulated in the dopamine synapses in the neostriatum.

Glutamatergic inputs in the neostriatum originate from the cerebral cortex, the thalamus, the hippocampus and the amygdala (Rebio et al., 1979; Fonnum et al., 1981; Wilson, 1983; Yoshikawa et al., 1991; Fuller et al., 1984). An increase in glutamate release in response to cocaine or methamphetamine challenge suggests that more glutamate is present in the neostriatum of the sensitized animal. However, it is not clear whether glutamatergic afferents from the 4 different sources enhanced glutamate release. Increase in glutamate release may result from one or more than one glutamatergic sources to the neostriatum. Thus, lesion studies are needed in the future to clarify the sources of increase in glutamate release in the sensitized animals. Moreover, although glutamate release from the corticostriatal projection is regulated by D2 dopamine receptors that are located on the terminals, whether glutamate release from other different sources is also affected by dopamine in the neostriatum needs to be clarified.

How Changes in Dopamine and Glutamate in Response to METH and Cocaine Injections Might Affect Locomotor Activity

In response to cocaine challenge, dopamine release was not increased in both the terminal and cell body regions. However, glutamate release in response to cocaine challenge was increased in the terminal and cell body regions. Augmentation in glutamate release in the striatum inhibits GABAergic neurons in the SNr, thus in turn

relieving its inhibition on the thalamus and increasing locomotor activity. An increase in glutamate release in the dopamine cell body region may excite dopamine neurons through its action on NMDA glutamatergic receptors (Overton and Clark, 1992; Johnson et al., 1992), consequently increasing the firing rate of dopamine neurons and leading to increase in dopamine release in the terminal regions. The latter may cause hyper-locomotor activity.

In response to METH challenge, dopamine release did not show significant increase in all the regions examined except for the NAc. In contrast, glutamate release was increased in response to METH challenge in all the regions examined except for the NAc. It seems likely that release of dopamine and glutamate in the neostriatum compensates for each other under METH challenge treatment. An increase in glutamate efflux in the caudate-putamen inhibits neurons in the SNr and leads to an increase in locomotor activity. An increase in dopamine release in the NAc activates the direct pathway and inhibits the indirect pathway, which also produces hyperlocomotor activity.

Morphological Data Indicating How Methamphetamine and Cocaine Treatments may Affect the Direct and Indirect Pathways

1) Fewer DA boutons were found in sensitized animals in the striatum after treatment with methamphetamine for about 2 weeks (Ihara, 1986). These morphological changes probably represent a high capability of nerve cells to reorganize synaptic

connections under methamphetamine treatment. However, they could not be related uniquely to behavioral hypersensivity.

2) Acute administration of METH and other amphetamine analogs such as P-chloroamphetamine and 3,4-methylenedioxymethamphetamine caused a dose-related decrease in [3H] dopamine uptake into striatal synaptosomes (Metzger et al, 1998). However, in the same study, acute and chronic administration of cocaine had no effect on [3H] dopamine transport into striatal synaptosomes.

3) Immunocytochemistry studies found that the density of 5-HT axons was selectively decreased either 4 hours or 2 weeks after high dose of METH administration (Axt and Molliver, 1991). However, the long-lasting axon loss only occurs in a fraction of animals.

4) Positron Emission Tomography (PET) studies in human methamphetamine abusers found that dopamine transporter (DAT) density were significantly decreased in the caudate nucleus. The decrease in DAT suggests that loss of DAT or loss of dopamine terminals in METH abusers, which may increase the risk for the development of Parkinson's disease (Una et al, 1998). Similarly, PET studies in baboons showed that striatal DAT density was significantly decreased 2-3 weeks after METH treatments (Villemagne et al, 1998). Reduction in DAT was also associated with decreases in DA determined in vitro. The results suggest that reduction in striatal DAT caused by METH is long lasting.

5) Chronic administration of cocaine caused reduction in the levels of 3 major neurofilament proteins in the VTA (Beitner-Johnson et al., 1992). The neurofilament proteins are components of the cytoskeleton and are involved in

axonal transport. Reduced amount of the neurofilament proteins may lead to a decrease in the transportation of tyrosine hydroxylase from the VTA to the dopaminergic terminals in the NAc, which eventually results in a reduction in dopamine synthesis and release in the NAc.

In summary, administration of psychomotorstimulant methamphetamine and cocaine produces a variety of alterations in the striatum. Although there are no direct correction between changes in locomotor activity in response to METH and cocaine administration and the morphological changes mentioned above, the drug-induced changes might play a role in behavioral sensitization. More researches are needed to study the involvement of morphological alteration in sensitized animals.

The Possible Molecular Mechanisms Underlying Behavioral Sensitization

Behavioral sensitization to METH and cocaine may be linked to changes in the activity of signaling components downstream of the dopamine and other monoamine receptors. A requirement for new gene expression during the development of sensitization is consistent with the observation that cocaine exposure results in induction of several immediate early genes such as c-Fos and FRA (Turgeon et al., 1998).

D1 dopamine receptors are coupled to the stimulatory G protein, which stimulates cAMP formation, whereas D2 dopamine receptors are linked to inhibitory G protein, which inhibits cAMP formation (Kebabian and Calne, 1979). Changes in dopamine release in response to psychomotorstimulant METH and cocaine injection

possibly cause changes in the cAMP second-messager and protein-phosphorylation systems in the postsynaptic neurons (Self and Nestler, 1994). Although D1 and D2 dopamine receptors may also utilize other second-messenger systems (Andersen et al, 1990), G protein-cAMP system may play an important role in the expression of behavioral sensitization to METH and cocaine.

Self-administration studies found that intra-NAc injection of PTX, which irreversibly inactivates Gi and Go proteins, reduced cocaine self-administration (Self and Nestler, 1994). Moreover, intra-NAc PTX injection caused rightward shifts in cocaine self-administration dose-response curves, suggesting that PTX antagonize the reinforcement effect of cocaine. On the other hand, CTX, which irreversibly activates Gs proteins, leads to increases in cocaine self-administration (Self and Nestler, 1994). Either PTX or CTX produces increase in cAMP levels in the affected neurons. These findings provide evidence to support the view that exposure to cocaine alters intracellular cAMP levels, which may result in changes in intracellular gene expression. Further studies are needed to identify genes that are involved in behavioral sensitization and addiction.

CHAPTER VII

References

Abarca, J., Gysling, K., Roth, R. H. and Bustos, G. (1995) Changes in extracellular levels of glutamate and aspartate in rat substantia nigra induced by dopamine receptor ligands: in vivo microdialysis studies, Neurochm. Res., 20 159-169.

Ackerman, J. M. and White, F. J. (1990) A10 somatodendritic dopamine autoreceptor sensitivity following withdrawal from repeated cocaine treatment, Neurosci. Lett., 117 181-184

Akimoto, K., Hamamura, T. and Otsuki, S. (1989) Subchronic cocaine treatment enhances cocaine-induced dopamine efflux, studied by in vivo intracerebral dialysis, Brain Res., 490 339-344.

Akimoto, K., Hamamura, T., Kazahaya, Y., Akiyama, K., and Otsuki, S. (1990) Enhanced extracellular dopamine level may be the fundamental neuropharmacological basis of cross-behavioral sensitization between methamphetamine and cocaine-an in vivo dialysis study in freely moving rats. Brain Res., 507 344-346.

Albin, R. L., Young, A. B. and Penney, J. B. (1989) The functional anatomy of basal ganglia disorders, Trends in Neuroscience, 12, 366-375.

Alburges, M. E., Narang, N., and Wamsley, J. K. (1993a) Alterations in dopaminergic receptor system after chronic administration of cocaine, Synapse, 14 314-317

Alburges, M. E., Anderson, J. J., Chase, T. N. and Engber, T. M. (1993b) Substance P increase release of acetylcholine in the dorsal striatum of freely moving rats, Brain Res., 623 189-194.

Altar, C.A. and Hauser, K. (1987) Topography of substantia nigra innervation by D1 receptor-containing striatal neurons, Brain Res., 410, 1-11.

Angulo, J. (1992) Involvement of dopamine D1 and D2 receptors in the regulation of proenkephalin mRNA abundance in the striatum and accumbens of the rat brain. J. Neurochem., 58 1104-1109.

Anderson, J. J., Chase, T. N., Engber, T. M. (1993) Substance P increases release of acetylcholine in the dorsal striatum of freely moving rats. Brain Res., 623 189-194.

Angulo, J. A., Cadet, J. L., Woolley, C., Suber, F., and McEwen, B. S. (1990) Effect of chronic typical and atypical neuroleptic treatment on proenkephalin mRNA levels in the striatum and nucleus accumbens of the rat. J. Neurochem., 54 1889-1894.

Angulo, J. A. and McEwen, B. S. (1994) Molecular aspects of neuropeptide regulation and function in the corpus striatum and nucleus accumbens. Brain Res. Rev., 19 1-28.

Araneda, R. and Bustos, G. (1989) Modulation of dendritic release of dopamine by N-methyl-D-aspartate receptors in rat substantia nigra. J. Neurochem. 52 962-970.

Arbuthnott, G. W., Fairbrother, I. S. and Butcher, S. P. (1990) Dopamine release and metabolism in the rat striatum: An analysis by in vivo brain microdialysis, Pharmacol. Ther., 48 281-293.

Axt, K. J., Molliver, M. E. (1991) Immunocytochemical evidence for methamphetamine-induced serotonergic axon loss in the rat brain. Synapse 9 302-313.

Azzaro A. and Rutledge C. O. (1973) Selectivity of release of norepinephrine, dopamine and 5-hydroxy-tryptamien by amphetamine in various regions of rat brain. Biochem. Pharmacol., 22 2801-2813.

Bain, G. T., Kornetsky, C. (1987) Naloxone attenuation of the effect of cocaine on rewarding brain stimulation. Life Sci., 40 1119-1125.

Bain, G. T., Kornetsky, C. (1989) Ethanol oral self-administration and rewarding brain stimulation. Alcohol., 6 499-503.

Bannon, M., Lee. J. M., Giraud, P., Affolter, H.-U. and Bonner, T. (1986) Dopamine antagonist haloperidol decreases substance P, substance K, and preprotachykinin mRNAs in rat striatonigral neurons. J. Biol. Chem., 261 6640-6644.

Baruch, P., Petit, F., Artaud, F., Basbeits, L., Godeheu, G., Cheramy, A., and Glowinski, J. (1986) Effects of tachykinins on dopamine release in the striatum: in vitro and in vivo studies, Substance P and Neurokinins. Springer-Verlag, 308-310.

Beckstead, R. M. (1979) An autoradiographic examination of cortical and subcortical projections of the mediodorsal-projection (prefrontal) cortex in the rat. J. Comp. Neurol., 184 43-62.

Beckstead, R. M. (1984) The thalamostriatal projection in the cat. J. Comp. Neural., 223 313-346.

Beiner-Johnson, D. and Nestler, E. J. (1991) Morphine and cocaine exert common chronic actions on tyrosine hydroxylase in dopaminergic brain reward regions. J. Neurochem., 57 344-347

Beiner-Johnson, D., Guitart, X., Nestler, E. J. (1992) Neurofilament proteins and the mesolimbic dopamine system: common regulation by chronic morphine and chronic cocaine in the rat ventral tegmental area. J. Neurochem., 61. 1766-1773.

Bjorklund, A. and Lindvall, O. (1975) Dopamine in dendrites of substantia nigra neurons. Suggestions for role in dendritic terminals, Brian Res., 83 531-537.

Bjorklund, A. and Lindvall, O. (1984) In: chemical neuroanatomy: classical transmitters in the CNS, Part 1. A. Bjorklund and O. lindvall, Eds. Elsevier, Amsterdam, pp. 55-122.

Bolam, J.P. and Smith, Y. (1990) The GABA and substance P inputs to dopaminergic neurons in the substantia nigra of the rat, Brain Res. 529 57-78.

Bourdelais, A. and Kalivas, P.W. (1991) High sensitivity HPLC assay for GABA in brain dialysis studies. J. Neurosci. Methods, 39 115-121.

Bouyer, J. J., Park, D. H., John, T. H., Pichel, V. M. (1984) Chemical and structural analysis of the relation between cortical inputs and tyrosine hydroxylase-containing terminals in rat neostriatum. Brain Res., 302 267-275.

Breiter, H. C., Gollub, R. L., Weisskoff, R. M., Kennedy, D. N., Makris, N., Berke, J. D., Goodman, J. M., Kantor, H. L., Gastfriend, D. R., Riorden, J. P., Mathew, R. T., Rosen, B. R. and Hyman, S. E. (1997) Acute effects of cocaine on human brain activity and emotion. Neuron, 19 591-611.

Cador, M., Dulluc, J., Moremde, P. (1993) Modulation of the locomotor response to amphetamine by corticosterone. Neuroscience 56, 981-988.

Carlson, J. H., Bergstrom, D. A., Demo, S. D., and Walters, J. R. (1990) Nigrostriatal lesion alters neurophysiological responses to selective and nonselective D1 and D2 dopamine agonists in rat globus pallidus. Synapse, 5 83-93.

Calabresi, P., Mercuri, N. B., Stefani, A., Bernardi, G. (1990) Synaptic and intrinsic control of membrane excitability of neostriatal neurons. I. An *in vivo* analysis. J. Neurophysisol., 63 651-662.

Carlsson, M.L., and Carlsson, A. (1989) The NMDA antagonist MK-801 causes marked locomotor stimulation in monoamine-depleted mice. J. Neural Transm., 75 221-226.

Carman, J. B., Cowan, W. M. and Powell, T. P. S. (1963) The organization of the corticostriate connections in the rabbit. Brain, 86 525-562.

Carman, J. B., Cowan, W. M. and Powell, T. P. S. and Webster, K. E. (1965) A bilateral corticostriate projection. J. Neurol. Neurosurg. Psychoat., 28 71-77.

Carter, C. J. (1982) Topographical distribution of possible glutamatergic pathways from the frontal cortex to the striatum and substantia nigra in rats. Neuropharm., 21 379-383.

Castaneda, E., Becker, J. B. and Robinson T. E. (1988) The long -term effects of repeated amphetamine treatment in vivo on amphetamine, KCl and electrical stimulation evoked striatal dopamine release in vitro. Life Science, 42 2447-2456.

Chang, H.T. (1988) Substance P - dopamine relationship in the rat substantia nigra: a light and electron microscopy study of double immunocytochemically labeled materials. Brain Res., 448 391-396.

Chen, J. Van Praag, H. M. and Gardner, E. L. (1991) Activation of 5-HT3 receptor by 1-phenylbiguanide increases dopamine release in the rat nucleus accumbens. Brain Res., 543 354-357.

Chevalier, G., Vacher, S., Deniau, J. M., Desban, M. (1985) Disinhibition as a basic process in the expression of striatal functions. I. The striato-nigral influence on tecto-spinal/tecto-diencephalic neurons. Brain Res., 334 215-226.

Clarke, P. B. S., Hommer, D. W., Pert, A., Skirboll, L. R. (1985) Electrophysiological actions of nicotine on substantia nigra single units. Br. J. Pharmacol., 85 827-835.

Clark, D., and White, F. J. (1987) Review: D1 dopamine receptor-the search for a function: A critical evaluation of the D1/D2 dopamine receptor classification and its functional implications. Synapse, 1 347-388.

Clow, D. W., and Jhamandas, K. (1989) Characterization of L-glutamate action on the release of endogenous dopamine from the rat caudate-putamen. J. Pharmacol. Exp. Ther., 248 722-728.

Cole, B. J., Cador, M., Stinus, L., Rivier, C., Rivier, J., Vale, W., Moal, M. L., Koob, G. F. (1990) Critical role of the hypothalamic pituitary adrenal axis in amphetamine-induced sensitization of behavior. Life Sciences, 47 1715-1720.

Connell, P. H. (1958) Amphetamine psychosis, Maudsley Monograph No. 5, Chapman and Hall, ltd., London.

Corbett, D., Fox, E., Milner, P. M. (1982) Fiber pathways associated with cerebellar self-stimulation in the rat: a retrograde and anterograde tracing study. Behav. Brain Res., 6 167-184.

Corbett, D., Wise, R. A. (1980) Intracranial self-stimulation in relation to the ascending dopaminergic systems of the midbrain: a moveable electrode mapping study. Brain Res., 185 1-15.

Cowan, W. M. and Powell, T. P. S. (1966) Striatopallidal projection in the monkey. J. Neurol. Neurosurg. Psychiat., 29 426-439.

Crawley, J. N. (1989) Microinjection of cholecystokinin into the rat ventral tegmental area potentiates dopamine-induced hypolocomotion. Synapse, 3 346-355.

Crawley, J. N. (1991) Cholecystokinin-dopamine interactions. Trends Pharmacol. Sci., 12 232-236.

Crawley, J. N., Stivers, J. A., Blumstein, L. K. and Paul, S. M. (1985) Cholecystokinin potentiates dopamine-mediated behaviors: Evidence for modulation specific to a site of coexistence. J. Neurosci., 5 1972-1983.

Crease, I. and Iversen, S. D. (1975) The pharmacological and anatomical substrates of the amphetamine response in the rat. Brain Res., 83 419-436.

Cunningham, K. A., Paris, J. M. and Goeders, N. E. (1992a) Chronic cocaine enhances serotonin autoregulation and serotonin uptake binding. Synapse, 11 112-123.

Cunningham, K. A., Paris, J. M. and Goeders, N. E. (1992b) Serotonin neurotransmission in cocaine sensitization. Annals of the New York Academy of Sciences, 654 117-127.

Dam, T.-V., Escher, E. and Quirion, R. (1990) Visualization of neurokinin-3 receptor sites in rat brain using the highly selective ligand [^{3}H] senktide. Brain Res., 506 175-179.

Dam, T.-V., Martinelli, B. and Quirion, R. (1990) Autoradiographic distribution of brain neurokinin-1 / substance P receptors using a highly selective ligand [^{3}H]-[Sar^9, Met(O$_2$)^{11}]-substance P. Brain Res., 531 333-337.

Das, G., and Laddu, A. (1993) Cocaine: Friend or foe? (Part I). Int. J. Clin. Pharmacol. Ther. Toxicol., 31 449-455.

Deniau, J. M. and Chevalier, G. (1985) Disinhibition as a basic process in the expression of striatal functions. II. The striato-nigral influence on thalamocortical cells of the ventromedial thalamic nucleus. Brain Res., 334 227-233.

Deschenes, R., Haun, R., Funckes, C., and Dixon, J. (1985) A gene encoding rat cholecystokinin. J. Biol. Chem., 260 1280-1286.

Devine, D. P., Leone, P., Pocock, D., Wise, R. A. (1993) Differential involvement of ventral tegmental *mu, delta,* and *kappa* opioid receptors in modulation of basal mesolimbic dopamine release: in vivo microdialysis studies. J. Pharmacol. Exp. Ther., 266 1236-1246.

Dougherty, G.G, Jr. and Ellinwood, E. H., Jr. (1981) Chronic d-amphetamine in nucleus accumbens: lack of tolerance of reverse tolerance of locomotor activity, Life Sci., 28 2295.

Ellinwood, E. H. (1972) 'Amphetamine psychosis: individuals, settings, and sequences'. in Current Concepts on Amphetamine Abuse (Eds. E. H. Ellinwood and S. Cohen), U.S. Government Printing Office, Washington, D.C.

Esposito, R., Kornetsky, C. (1977) Morphine lowering of self-stimulation thresholds: lack of tolerance with long-term administration. Science, 195 189-191.

Esposito, R., Perry, W., Kornetsky, C. (1980) Effects of d-amphetamine and Naloxone on brain stimulation reward. Psychopharm., 69 187-191.

Fallon, J. H.. (1978) Collateralization of monoamine neurons: mesotelencephalin dopamine projections to caudate, septum, and frontal cortex. J. Neuro. Sci., 1 1361-1368.

Fallon, J. H. and Moore, R. Y. (1978) Catecholamine innervation of the basal forebrain: IV. Topography of the dopamine projection to the basal forebrain and neostriatum. J. Comp. Neurol., 180 545-580.

Fallon, J. H. and Loughlin, S. E. (1982). Monoamine innervation of the basal forebrain: Forebrain collateralization. Brain Res. Bull., 9 295-307.

Farfel, G. M., Kleven, M. S., Woolverton, W. L., Seiden, L. S., and Perry, B. D. (1992) Effects of repeated injections of cocaine on catecholamine receptor binding sites, dopamine transporter binding sites and behavior in rhesus monkey. Brain Res., 578 235-239.

Flaherty, A. W. and Graybiel, A. M. (1994) Input-output organization of the sensorimotor striatum in the squirrel monkey. J. Neuro. Sci., 14 599-610.

Fonnum, F., Soreide, A., Krale, I., Walker, J., and Walaas, I. (1981) Glutamate in cortical fibers. Adv. Biochem. Psychopharmacol., 25 29-42.

Fouriezos, G., Wise, R. A. (1976) Pimozide-induced extinction of intracranial self-stimulation: response patterns rule out motor or performance deficits. Brain Res., 103 377-380.

Freeman, A., Chiodo, L., Lentz, S., Wade, K., and Bannon, M. (1991) Release of cholecystokinin from rat midbrain slices and modulatory effect of D2 dopamine receptor stimulation. Brain Res., 555 281-287.

Fuller, T. A., Russchen, F. T. and Price, J. L. (1984) Presumptive glutamergic/aspartertic cells projecting to the olfactory cortex. Soc. Neurosci. Abst. 10, 227.

Gariano, R. F. and Groves, P. M. (1988) dopamine neurons by stimulation of the medial prefrontal and anterior cingulate cortices. Burst firing induced in midbrain Brain Res., 462 194-198.

Gawin, F. H. and Ellinwood, E. H., Jr. (1988) Cocaine and other stimulants: actions, abuse and treatment. N. Engl. J. Med., 318 1173.

Gerfen, C. R., Staines, W. A., Arbuthnot, G. W. and Fibiger, H. C. (1982) Crossed connections of the substantia nigra in the rat. J. Comp. Neurol., 207 283-303.

Gerfen, C. R. (1984) The neostriatal mosaic: Compartmentalization of corticostriatal input and striatonigral output system. Nature, 311 461-464.

Gerfen, C. R and W. S. Young III (1988) Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. Brain Res., 460 161-163.

Gerfen, C. R. (1991) Substance P (neurokinin-1) receptor mRNA is selectively expressed in cholinergic neurons in the striatum and basal forebrain. Brain Res., 566 165-170.

Gerfen, C. R., McGinty, J. F. and Young, W. S. III (1991) dopamine differentially regulates dynorphin, substance P and enkephalin expression in striatal neurons: in situ hybridization histochemical analysis. J. Neurosci., 11 1016-1031.

Gerfen, C. R., (1992) The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. Annu. Rev. Neurosci., 15 285.

Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J., JR, Sibley, D. R. (1990) D1 and D2 Dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science, 250 1429-1432.

Giovannini M. G., Camilli F., Mundula A., and Pepeu, G. (1994) Glutamatergic regulation of acetylcholine output in different brain regions: a microdialysis study in the rat. Neurochem. Int., 25 23-26.

Goeders, N. E. and Smith, J. E. (1983) Cortical dopaminergic involvement in cocaine reinforcement. Science, 221 773.

Goeders, N. E., Dworkin, S. I., and Smith, J. E. (1986) Neuropharmachological assessment of cocaine self-administration into the medial prefrontal cortex. Pharmacol. Biochem. Behav., 24 1429.

Goldman, P. S. and Nauta, W. J. H. (1977) An intricately patterned prefronto-caudate projection in the rhesus monkey. J. Comp. Neuro., 171 369-386.

Gonon, F. G. (1988) Nonlinear relationship between impulse flow and dopamine released by rat midbrain dopaminergic neurons as studied by in vivo electrochemistry. Neuroscience, 24 19-28.

Godukhin, O. V., Budantsev, A. Y., Selifanova, O. V., and Agapova, V. N. (1984) Effect of cholinomimetics on the release and uptake of L-[3H] glutamic acid in rat neostriatum. Cell. Mol. Neurobiol., 4 117-124.

Greenamyre, J. T. and Young, A. B. (1989) Synaptic localization of striatal NMDA, quisqualate and kainate receptors. Neurosci. Lett., 101 133-137.

Griffith, J. D., Cavanaugh, J. H. and Oates, J. A. (1970) 'Psychosis induced by the administration of d-amphetamine to human volunteers', in Psychotomimentic drugs (Ed. D. H. Efron), Raven Press, New York.

Grima, B., Lamoroux, A., Blanot, F., Biguet, N., and Mallet, J. (1985) Complete coding sequence of rat tyrosine hydroxylase mRNA . Proc. Natl. Acad. Sci. U.S.A., 82 617-621.

Grinspoon, L., and Hedblom, P. (1975) The speed culture: Amphetamine use and abuse in America. Harvard University Press, Cambridge MA.

Guevara Guzman, R., Kendrick, L. M., and Emson, P. C. (1993) Effect of substance P on acetylcholine and dopamine release in the rat striatum: a microdialysis study. Brain Res., 622 147-154.

Graham, J. H., and Porrino, L. J. (1993) Neuroanatomical basis of cocaine self-administration. The Neurobiology of Cocaine, cellular and molecular mechanisms.

Gunne, L. (1977) 'Effects of amphetamines in humans', in Drug Addiction, II (Ed. J. R. Martin), Springer-Verlag, New York.

Heath, R. G. (1963) Intracranial self-stimulation in man. Science, 140 394-396.

Heath, R. G. (1972) Pleasure and brain activity in man. J. Nerv. Ment. Dis., 154 3-18.

Helke, C.J., Krause, J.E., Mantyh, P.W., Couture,R. and BannonJ. (1990) Diversity in mammalian tachykinin peptidergic neurons: multiple peptides, receptors, and regulatory mechanisms. FASEB J., 4 1606-1615.

Henry, D. J., Greene, M. A., and White, F. J. (1989) Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: repeated administration. J. Pharmacol. Exp. Ther., 251 833-384.

Henry, D. and White, F. (1991) Repeated cocaine administration causes persistent enhancement of D1 dopamine receptor sensitivity within the rat nucleus accumbens. J. Pharmacol. Exp. Ther., 258 882-890.

Herkenham, M. (1987) Mismatches between neurotransmitter and receptor localizations in brain: observations and implications. Neurosci., 23 1-38.

Herrling, P.L, and Hull, C.D. (1980) Iontophoretically applied dopamine depolarizes and hyperpolarizes the membrane of cat caudate neurons. Brain Res. 192 441-462.

Hiroyuki, N., Tadaaki, H., Hideaki, T., Seiichi, I., Shigetada, N. (1983) Nucleotide sequences of cloned cDNAs for two types of bovine brain substance P precursor. Nature, 306 32-36.

Hokfelt, T., Johansson, O. and Goldstein, M. (1984) Chemical neuroanatomy of the brain. Science, 225 1326-1335.

Hokfelt, T., Reid, M., Herrera-Marschitz, M., Ungerstedt, U., Terenius, L., Hakanson, R., Feng, D. M. and Folkers, K. (1991) Tachykinins and related peptides in the substantia nigra and neostriatum. Substance P and Related Peptides. The New York Academy of Science, 192-197.

Hong, J. S., Yang, T., Gillin, J. and Costa, E. (1980) Effects of long-term administration of antipsychotic drugs on enkephalinergic neurons. Adv. Biochem. Psychopharmacol, 24 223-232.

Hooks, M. S., Jones, G. H., Liem, B. J, Justice J. B. (1992) Sensitization and individual differences to IP amphetamine, cocaine, or caffeine following repeated intracranial amphetamine infusions. Pharmacol. Biochem. Behav., 43 815-823.

Hooks, M. S. and Kalivas, P. W. (1994) Involvement of dopamine and excitatory amino acid transmission in novelty-induced motor activity. J. Pharmacol. Exp. Ther., 269 976-988.

Horn, A. S. (1990) Dopamine uptake: A review of progress in the last decade. Pro. Neurobiol., 34 387-400.

Hornykiewicz, O. (1981) Brain neurotransmitter changes in Parkinson's disease. In Movement Disorders, C. D. Marsden and S. Fahn (Eds.), Elsevier/Butterworths, London, PP.4-58.

Hubner, C. B. and Koob, G.F. (1990) The ventral pallidum plays a role in mediating cocaine and heroin self-administration in the rat. Brain Research, 508 20.

Hubner, C. B., Kornetsky, C. (1992) Heroin, 6-acetylmorphine and morphine effects on threshold for rewarding and aversive brain stimulation. J. Pharmacol. Exp. Ther., 260 562-567.

Hurd, Y. L., Weiss, F., Koob, G. F., Anden, N-E. and Ungerstedt, U. (1989) Cocaine reinforcement and extracellular dopamine overflow in rat nucleus accumbens: an in vivo microdialysis study. Brain Res., 498 199-203.

Ihara, Y. Sato, M. Otsuki, S, Kaiya, H, Namba, M. (1986) Morphological changes in rat striatal boutons after chronic methamphetamine and haloperidol treatment. Neurosci Res., 3 403-410.

Izenwasser, S. and Cox, B. M. (1992) Inhibition of dopamine uptake by cocaine and nicotine: tolerance to chronic treatments. Brain Res., 573 119-125.

James, T. A., and Starr, M. S. (1977) Behavioral and biochemical effects of substance P injection and expression of drug-and stress-induced sensitization of motor activity. Brain Res. Rev., 9 573-587.

Jayaraman, A. (1984) Thalamostriate projections-an overview. In J. S. MckKenzie, R. E. Kemm and L. N. Wilcock (eds), The basal ganglia: structure and function, PP. 69-86. Plenum Press, New York.

Jessell, T. M. (1978) Substance P release from the rat substantia nigra. Brain Res., 151 469-478.

Johanson, C. E. and Fischman, M. W. (1989) The Pharmacology of cocaine related to its abuse. Pharmacol. Rev., 41 3-52.

Johnson, S. W., Seutin, V. and North, R. A. (1992) Burst firing in dopamine neurons induced by N-methy-D-aspartate: role of electrogenic sodium pump. Science, 258. 665-667.

Jonsson, L. E. and Gunne. L. M. (1970) 'Clinical studies of amphetamine psychosis', in Amphetamines and Related compounds (Eds. E. Costa and S. Garattini), Raven Press, New York.

Joyce, E. M. and Jversen, S. D. (1979) The effect of morphine applied locally to mesencephalic dopamine cell bodies on spontaneous motor activity in the rat. Neurosci. Lett., 14 207-212.

Kaddis, F. G., Wallace, L. J., and Uretsky, H. J. (1993) AMPA/kainate antagonists in the nucleus accumbens inhibit locomotor stimulatory response to cocaine and dopamine agonists. Pharmacol. Biochem. Behav., 46 703-708.

Karler, R., Calder, L. D., Chaudhry, I. A. and Turkanis, S. A. (1989) Blockade of 'reverse tolerance' to cocaine and amphetamine by MK-801. Life Sciences, 45 599-606.

Karler, R., Calder, L. D. and Turkanis, S. A. (1991) DNQX blockade of amphetamine behavioral sensitization. Brain Res. 552 295-300.

Kalivas, P. W. (1985) Interactions between neuropeptides and dopamine neurons in the ventromedial mesencephalon. Neurosci. Behav. Rev., 9 573-587.

Kalivas, P. W. (1993) Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. Brain Res. Rev., 18 75-113.

Kalivas, P. W. and Duffy, P. (1988) Effects of daily cocaine and morphine treatment on somatodendritic and terminal field dopamine release. Journal of Neurochem., 50 1498-1504.

Kalivas, P. W. and Alesdatter J. E. (1993) Involvement of NMDA receptor stimulation in the VTA and amygdala in behavioral sensitization to cocaine. J. Pharmacol. Exp. Ther., 267 486-495.

Kalivas, P. W. and Duffy, P. (1990) The effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. Synapse, 5 48-58.

Kalivas, P. W. and Stewart, J. (1991) Dopamine transmission in the initiation and expression of drug- and stress- induced sensitization of motor activity. Brain Res. Rev., 16 223-224.

Kalivas, P. W., Weber, B. (1988) Amphetamine injection into the A10 dopamine region sensitizes rats to peripheral amphetamine and cocaine. J Pharmacol. Exp. Ther., 245 1095-1102.

Kalivas, P. W. (1993) Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. Brain Res. Rev., 18 75-113.

Kalivas, P. W. and Duffy, P. (1993a) Time course of extracellular dopamine and behavioral sensitization to cocaine. I. dopamine axon terminals. J. Neurosci., 13 276-284.

Kalivas, P. W. and Duffy, P. (1993b) Time course of extracellular dopamine and behavioral sensitization to cocaine. II. Dopamine perikarya. J. Neurosci., 13 266-275.

Kalivas, P. W. and Duffy, P. (1995) D1 receptors modulate glutamate transmission in the ventral tegmental area. J. Neurosci., 15 5379-5388.

Kalivas, P. W., Duffy, P. and Barrow, J. (1989) Regulation of the mesocorticolimbic dopamine system by glutamic acid receptor subtypes. J. Pharmacol. Exp. Ther., 251 378-387.

Karler, R., Calder, L. D., and Turkanis, S. A. (1991). DNQX blockade of amphetamine behavioral sensitization. Brain Res., 552 295-300.

Kashihara, K., Hamamura, T., Okumura, K. and Otsuki, S. (1990) Effect of MK-801 on endogenous dopamine release in vivo. Brain Res., 528 80-82.

Kawaguchi, Y., Charles, J. W., and Piers, C. E. (1990) Projection subtypes of rat neostriatal matrix cells revealed by intracellular injection of biocytin. J. Neurosci., 10 3421-3438.

Kawaguchi, Y., Charles, J. W., Augood, S. J. and Piers, C. E. (1995) Striatal interneuron: chemical, physiological and morphological characterization. Trends in Neurosci., 18 527-535.

Kazahaya, Y., Akimoto, K., and Saburo, O. (1989) Subchronic methamphetamine treatment enhances methamphetamine-or cocaine-induced dopamine efflux in vivo. Biol. Psychiatry., 25 903-912.

Kebabian, J. W. and Calne, D. B. (1979) Multiple receptors for dopamine. Nature 277 93-96.

Kelley, A. E., Domesick, V. B. and Nauta, W. J. H. (1982) The amygdalostriatal projection in the rat-an anatomical study by anterograde and retrograde tracing methods. Neurosci., 7 615-630.

Kelley P. H. and Iversen S. D. (1975) Selective 6-OHDA-induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant-induced locomotor activity in rats. Eur. J. Pharmacol., 186 1-8.

Kelly, A., Cador, M. and Stinus, L. (1985) Behavioral analysis of the effect of substance P injected into the ventral mensencephalon on investigatory and spontaneous motor behavior in the rat. Psychopharmacol., 85 37-46.

Kita, H. and Kitai, S. T. (1988) Glutamate decarboxylase immunoreactive neurons in rat neostriatum: their morphological types and populations. Brain Res., 447 346-352.

Kitai, S. T., Koscis, J. D., Preston, R. J., Sugimori, M. (1976) Monosynaptic inputs to caudate neurons identified by intracellular injection of horseradish peroxidase. Brain Res., 109 601-606.

Kitai, S. T., Sugimori, M., and Kocsis, J. (1976) Excitatory nature of dopamine in the nigro-caudate pathway. Exp. Brain Res., 24 351-363.

Kitai, S. T. (1981) Anatomy and physiology of the neostriatum. In G. DiChiara and G. L. Gessa (eds), GABA and the basal ganglia. Raven Press, New York.

Kiyama, H., McGowan, E., and Emson, P. (1991) Co-expression of cholecystokinin mRNA and tyrosine hydroxylase mRNA in populations of rat substantia nigra cells; a study using a combined radioactive and non-radioactive in situ hybridization procedure. Mol. Brain Res., 9 87-93.

Klockgether, T., and Turski, L. (1990) NMDA antagonists potentate antiparkinsonian actions of L-dopa in monoamine-depleted rats. Ann. Neurol., 28 539-546.

Koetter, R. (1994) Postsynaptic integration of glutamatergic and dopaminergic signals in the striatum. Prog. Neurobiol., 44 163-196.

Kolta, M. G., Shreve, P., De Souza, V. and Uretsky, N. J. (1985) Time course of the development of the enhanced behavioral and biochemical responses to amphetamine after pretreatment with amphetamine. Neuropharmachol., 24 823-829.

Kolta, M. G., Shreve, P and Uretsky, N. J. (1989) Effect of pretreatment with amphetamine on the interaction between amphetamine and dopamine neurons in the nucleus accumbens Neuropharmachol., 28 9-14.

Koob, G., and Bloom, F. (1988) Cellular and molecular mechanisms of drug dependence. Sci., 242 715-723.

Kopin, I. J. (1985) Catecholamine metabolism: Basic aspects and clinical significance. Pharmacol. Rev., 37 333-364.

Kornetsky, C. and Esposito, R. (1981) Reward and detection thresholds for brain stimulation: dissociation effects of cocaine. Brain Res., 209 496-500.

Kornetsky, C. and Esposito, R. (1979) Euphorigenic drugs: effects on the reward pathways of the brain. Fed. Proc., 38 2473-2476.

Krause, J., Chirgwin, J., Carter, M., Xu, Z. and Hershey, A. (1987) Three rat preprotachykinin mRNAs encode the neuropeptides substance P and neurokinin A. Proc. Natl. Acad. Sci. U. S. A., 84 881-885.

Krebs, M., Desce, J., Kemel, M., Gauchy, C., Godeheu, G., Cheramy, A. and Glowinski, J. (1991) Glutamatergic control of dopamine release in the rat striatum: evidence for presynaptic N-methyl-D-aspartate receptors on dopaminergic nerve terminals. J. Neurochem., 56 81-85.

Krettek, J. E. and Price, J. L. (1978) Amygdaloid projections to subcortical structures within the forebrain and brain stem in the rat and cat. J. Comp. Neurol., 178 225-254.

Lacey, M. G., Mercuri, N. B., and North, R. A. (1987) Dopamine acts on D2 receptors to increase potassium conductance in neurons of the rat substantia nigra zona compacta. J. Physiol. (Lond.) 392 397-416.

Lee, C.M., Campell, N.J., Williams, B.J. and Iversen, L.L. (1986) Multiple tachykinin binding sites in peripheral tissues and in brain. Eur. J. Pharmaco., 130 209-217.

Le Moal, M. and Simon, H. (1991) Mesocorticolimbic dopaminergic network: functional and regulatory roles. Physiol. Rev., 7 1155-1233.

Li, S. J., Jiang, H. K., Stachowiak, M. S., Hudson, P. M., Owyang, V., Nanry, K. Tilson, H. A. and Hong, J. S. (1990) Influence of nigrostriatal dopaminergic tone on the biosynthesis of dynorphin and enkephalin in rat striatum. Molecular Brain Res., 8 219-225.

Loughlin, S., and Fallon, J. (1984) Substantia nigra and ventral tegmental area projections to cortex: topography and collateralization. Neurosci., 11 425-435.

Maggi, C. A. (1994) The mammalian tachykinin receptors. Gen. Pharmac., 26 911-944.

Mansour, A., Meador-woodruff, J., Bunzow, J., Civelli, O., Akil, H. and Watson, S. (1990) Localization of dopamine D2 receptor mRNA and D1 and D2 receptor binding in situ hybridization-receptor autoradiographic analysis. J. Neurosci., 10 2587-2600.

Mansour, A., Meador-Woodruff, J.H., Healy, D. J., Kuehn, R., Zhou, Q-Y., Bunzow, J. R., Akil, H., Civelli, O. and Watson Jr., S. J. (1992) A comparison of D1 receptor binding and mRNA in rat brain using receptor autoradiographic and in situ hybridization techniques. Neurosci., 46 959-971.

Mantyh, P.W., Hunt, S.P. and Maggio, J. E. (1984) Substance P receptors: localization by light microscopic autoradiography in rat brain using [^{3}H] SP as the radioligand. Brain Res., 307 147-164.

Mantyh, P.W., Gates, T., Mantyh, C.R. and Maggio, J.E. (1989) Autoradiographic localization and characterization of tachykinin receptor binding sites in the rat brain and peripheral tissues. J. Neurosci., 9 258-279.

Maura, G., Giardi, A., and Raiteri, M. (1988) Release-regulating D2 dopamine receptors are located on striatal glutamatergic nerve terminals. J. Pharmacol. Exp. Ther., 247 680-684.

Maycox, P. R., Hell, J. W., and Jahn, R. (1990) Amino acid neurotransmission: Spotlight on synaptic vesicles. Trends Neurosci., 13 83-87.

Meador-Woodruff, J.H., Mansour, A., Healy, D. J., Kuehn, R., Zhou, Q-Y., Bunzow, J. R., Akil, H., Civelli, O. and Watson Jr., S. J. (1991) Comparison of the distributions of D1 and D2 dopamine receptor mRNA's in rat brain, Neuropsychopharmacol., 5 231-242.

Meiergerd, S. M., Hooks, S. M., Schenk, J. O. (1994a) The striatal transporter for dopamine in the rat may be kinetically up-regulated following 3 weeks of withdrawal from cocaine self-administration. J. Neurochem., 63 1277-1281.

Metzger, R. R., Hanson, G. R., Gibb, J. W., Fleckenstein, A. E. (1998) 3-4-methylenedioxymethamphetamine-induced acute changes in dopamine transporter function. Eur. J. Pharmacol., 349 205-210.

Meiergerd, S. M., McElvain, J. S., Schenk, J. O. (1994b) Effects of cocaine and repeated cocaine followed by withdrawal. Alterations of dopaminergic transporter turnover with no changes in kinetics of substrate recognition. Biochem. Pharmacol., 47 1627-1634.

Missale, C., Castelletti, L., Govoni, S., Spano, P. F., Trabuchi, M. and Hanbauer, I. (1985) Dopamine uptake is differentially regulated in rat striatum and nucleus accumbens. Journal of Neurochem., 45 51-56.

Mogenson, G. J. and Yang, C. R. (1991) The contribution of basal forebrain to limbic-motor integration and the mediation of motivation to action. In: The Basal forebrain. Anatomy to Function (Eds TC Napier, PW Kalivas and I Hanin), pp. 267-290. Plenum Press, New York.

Moore, K. E. (1978) Amphetamines: biochemical and behavioral actions in animals. In: Handbook of Psychopharmacology (Iversen, L. L., Iversen, S. D. and Snyder, S. H., Eds), pp.41-98. Plenum Press, New York.

Morari, M., O'Connar, W. T., Ungerstedt, U. and Fuxe, K. (1993) NMDA differentially regulates extracelllular dopamine, GABA, and glutamate levels in the dorsolateral neostriatum of the halothane-anesthetized rat: an in vivo microdialysis study. J. Neurochem., 60 1884-1893.

Muscat, R., Patel, J., Trout, S.J., Wieczorek, W., and Kruk, Z. L. (1993) dissociation of the effects of amphetamine and quinpirole on dopamine release in the nucleus accumbens following behavioral sensitization: an ex vivo voltammetric study, Behav. Pharmacol., 4 411-418.

National Institute on Drug Abuse. (1991) National Household Survey on Drug Abuse. Population Estimates, 1990. U. S. Dept. of Health and Human Services, Rockville, Maryland.

Nagatsu, T., Levitt, M., and Udenfriend, S. (1964) Tyrosine hydroxylase: The initial step in norepinephrine biosynthesis. J. Biol. Chem., 238 2910-2917.

Nelson, L. R. and Ellison, G. (1978) Enhanced stereotypes after repeated injections but not continuous amphetamines. Neuropharmachol. 17 1081-1084.

Ng, J. P., Hubert, G. W. and Justice, J. B. Jr. (1991) Increased stimulated release and uptake of dopamine in the nucleus accumbens after repeated cocaine administration as measured by in vivo voltammetry. J Neurochem., 56 1485-1492.

Nicholls, D.G. (1989) Release of glutamate, aspartate, and r-aminobutyric acid from isolated nerve terminals. J. Neurochem., 52 331-341.

Nicholls, D.G. and Attwell, D. (1990) The release and uptake of excitatory amino acids. Trends Pharmacol. Sci., 11 462-468.

Nicolaysen, L. C. and Justice, J. B. Jr. (1988) Effects of cocaine on release and uptake of dopamine in vivo: differentiation by mathematical modeling. Pharmacol. Biochem. Behav., 31 327-335.

Olpe. H. R. and Koella, W. P. (1977) Rotatory behavior in rats by inra-nigral application of substance P and an eledoisin fragment. Brain Res., 126 576-579.

Overton, P. and Clark, D. (1992) Iontophoretically administered drugs acting at the N-methyl-D-aspartate receptor modulate burst firing in A9 dopamine neurons in the rat. Synapse, 10 131-240.

Olds, J. (1956) Pleasure centers in the brain. Sci. Am., 195 105-116.

Olds, J., Olds, M. E. (1965) Drives, rewards, and the brain. In new directions in psychology, Ed. TM Newcombe, pp, 327-410. New York: Holt, Rinehart & Winston.

Olds, M. E., Olds, J. (1963) Approach-avoidance analysis of rat diencephalon. J. Comp. Neurol., 120 259-295.

Palkovits, M and Brownstein, M. (1988) Maps and guide to microdissection of the rat brain. Elsevier, New York.

Parsons, L. H. and Justice, J. B. Jr. (1993) Serotonin and dopamine sensitization in the nucleus accumbens, ventral tegmental area and dorsal raphe nucleus following repeated cocaine administration. J. Neurochem., 61 1611-1619.

Parsons, L. H., Smith, A. D. and Justice, J. B. Jr. (1991) Basal extracellular dopamine is decreased in the rat nucleus accumbens during abstinence from chronic cocaine. Synapse, 9 60-65.

Patrick, S. L., Thompson, T. L., Walker, J. M., and Patrick, R. L. (1991). Concomitant sensitization of amphetamine-induced behavioral stimulation and in vivo dopamine release from rat caudate nucleus. Brain Res., 538 343-346.

Paulson, P. E., Camp, D. M. and Robinson, T. E. (1991) Time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats. Psychopharmacol., 103 480-492.

Paulson, P. E., and Robinson, T. E. (1994) Relationship between circadian changes in spontaneous motor activity and dorsal versus ventral striatal dopamine neurotransmission assessed with on-line microdialysis. Behav. Neurosci., 108 624-635.

Paulson, P.E., and Robinson, T. E. (1995) Amphetamine-induced time-dependent sensitization of dopamine neurons an study in behaving rats. Synapse, 19 56-65.

Pellegrino, L. J., Pellegrino, A. S. and Cushman, A. J. (1979) A stereotaxic Atlas of the rat brain. Plenum Press, New York.

Peris, J., Boyson, S. J., Cass, W. A., Curella, P., Dwoskin, L. P., Larson, G., Lin, L-H., Yasuda, R. P. and Zahniser, N. R. (1990) Persistence of neurochemical

changes in dopamine systems after repeated cocaine administration. J. Pharm. Exper. Ther., 253 38-44.

Petersen, R. C. (1977) Cocaine: An overview. NINA Res. Monogr., 13 17-34.

Pettit H. O., Pan H. T., Parsons L. H., Justice J. B. Jr. (1990) Extracellular concentrations of cocaine and dopamine are enhanced during chronic cocaine administration. J. Neurochem., 55 798-804.

Pettit, F. And Glowinski, J. (1986) Stimulatory effect of substance P on the spontaneous release of newly synthesized [^{3}H] dopamine form rat striatal slices: a tetrodotoxin-sensitive process. Neuropharmacol., 25 1015-1021.

Piazza, P. V., Deminiere, J-M., Maccari, s., Mormede, P., Le Moal,M. and Simon, H. (1990a) Individual reactivity to novelty predicts provability of amphetamine self-administration. Behav. Pharmacol., 1 339-345.

Piazza, P. V., Deminiere, J. M., Lemoal, M. and Simon H. (1990b) Stress-and pharmacologically- induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. Brain Res., 514 22-26.

Pollack, A. E. and Wooten, G. F. (1992) D2 dopaminergic regulation of striatal preproenkephalin mRNA levels is mediated at least in part through cholinergic interneuron. Molecular Brain Res., 13 35-41.

Pierce, R. C., Duffy, P. and Kalivas, P. W. (1995) Sensitization to cocaine and dopamine autoreceptor subsensitivity in the nucleus accumbens. Synapse, 20 33-36.

Porceddu, M. L., Giorgi, O., Ongini, E., Mele, S. and Bibbio, G. (1980) 3H-SCH 23390 binding sites in the rat substantia nigra: evidence for a presynaptic localization and innervation by dopamine. Life Sci., 39 321-328.).

Post, R. M. (1980) Intermittent versus continuous stimulation: effect of time interval on the development of sensitization of tolerance. Life Sci., 26 1275-1282.

Pulvirenti, L., Swerdlow, N. R., and Koob, G. F. (1989) Microinjection of a glutamate antagonist into the nucleus accumbens reduces psychostimulant locomotion in rats. Neurosci. Lett., 103 213-218.

Rao, T. S., Kim, H. S., Lehmann, J., Martin, L. L., and Wood, P. L. (1990) Interactions of phencyclidine receptor agonist MK-801 with dopaminergic system: regional studies in the rat.. J. Neurochem., 54 1157-1162.

Rawlin, J. W. (1968) Street level abuse of amphetamines. In Amphetamine Abuse (J. R. Russo, Ed.), pp. 51-65. Thomas, Springfield, IL.

Reubi, J., Cuenod, M. (1979) Glutamate release in vitro from corticostriatal terminal. Brain Res., 176 185-188.

Ritz, M. C., Cone, E. J. and Kuhar, M. J. (1990) Cocaine inhibition of ligard binding at dopamine, norepinephrine and serotonin transporters: A structure-activity study. Life Sci., 46 635-645.

Roberts, D. C. S. and Koob, G. F. (1980) Extinction and recovery of cocaine self-administration following 6-hydrodopamine lesions of the nucleus accumbens. Pharmacol. Biochem. & Behav., 12 781-787.

Roberts, D. C. S. and Koob, G. F. (1982) Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. Pharmacol. Biochem. Behav., 17 901.

Roberts, P. J., McBean, G. J., Sharif, N. A. and Thomas, E. M. (1982) Striatal glutamatergic function: modifications following specific lesions. Brain Res., 235 83-91.

Roberts, P. J. and Sharif, N. A. (1978) Effects of L-glutamate and related amino acids upon the release of [3H] dopamine from rat striatal slices. Brain Res., 157 391-395.

Robinson T. E. and Becker, J. B. (1982) Behavioral sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from striatal tissue in vitro. Eur. J. Pharmacol., 85 253-254.

Robinson T.E. and Becker, J. B. (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. Brain Res. Rev., 11 157-198.

Robinson, T. E., Jurson, P. A., Bennett, J. A., and Bentgen, K. M. (1988) Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (+)-amphetamine: A microdialysis study in freely moving rats. Brain Res., 462 211-222.

Robledo, P. and Feger, J. (1990) Excitatory influence of rat subthalamic nucleus to substantia nigra pars reticulata and the pallidal complex: electrophysiological data. Brain Res., 518 47-54.

Romano, G., Shivers, B., Harlan, R., Howells, R. and Pfaff, D. (1987) Haloperidol increases proenkephalin mRNA levels in the caudate-putamen of the rat: a quantitative study at the cellular level using in *situ* hybridization. Mol. Brain Res., 2 33-41.

Rosecran, J. S., and Spitz., H. I. (1987) Cocaine reconceptualized: Historical overview. In cocaine Abuse (H. I. Spotz and J. S. Rosecran, Eds.), pp. 5-16. Brunner/Mazel, New York.

Rosen, H., Douglass, J. and Herbert, E. (1984) Isolation and characterization of the rat proenkephalin gene. J. Bio. Chem., 259 14309-14313.

Royce, G. J. (1978). Cells of origin of subcortical afferents to the caudate nucleus: A horseradish peroxidase study in the cat. Brain Res., 153 465-475.

Royce, G. J. (1982). Laminar origin of cortical neurons which project upon the caudate nucleus. A horseradish peroxidase investigation in the cat. J. Comp. Neurol., 205 8-29.

Russchen, F. T. and Price, J. L. (1984) Amygdalostriatal projections in the rat. Topographical organization and fiber morphology shown using the lectin PHA-L as an anterograde tracer. Neurosci. Lett., 47 15-22.

Sacaan, A. I., Bymaster, F. P., and Schoepp, D. D. (1992) Metabotropic glutamate receptor activation produces extrapyramidal motor system activation that is mediated by striatal dopamine. J. Neurochem., 59 245-251.

Schalling, M., Hokfelt, T., Wallace, B., Goldstein, M., Filer, D., Yamin, C., and Schlessinger, D. (1986) Tyrosine hydroxylase in rat brain and adrenal medulla: hybridization histochemistry with immunohistochemistry combined with retrograde tracing. Proc. Natl. Acad. Sci. U.S.A., 83 6208-6212.

Schalling, M., Friberg, K., Seroogy, K., Riederer, P., Bird, E., Schiffmann, S. N., Mailleux, P., Vanderhaeghen, J.-J., Kuga, S., Goldstein, M., Kitahama, K., Luppi, P. H., Jouvet, M., and Hokfelt, T. (1990) Analysis of expression of cholecystokinin in dopamine cells in the ventral mesencephalon of several species and in humans with schizophrenia. Proc. Natl. Acad. Sci. U.S.A., 87 8427-8431.

Scheel-Kruger J, Graestrup C, Nielson M, Golembiowski K, Mogilmicka E. (1977) Cocaine: discussion on the role of dopamine in the biochemical mechanism of action. In: Cocaine and other stimulants (Ellinwood E Jr, Kilby M, eds), 373-407. Yew York: Plenum.

Schmidt, W. J. (1986) Intrastriatal injection of d, 1-2-amino-5-phosphonovaleric acid (AP-5) induced sniffing that is antagonized by haloperidol and clozapine, Psychopharmacol., 90 123-130.

Schmidt, W. J., and Bury, D. (1988) Behavioral effects of N-methyl-D-aspartate in the anterodorsal striatum of the rat. Life. Sci., 43 545-549.

Segal, D. S., Geyer, M. A., and Schuckit, M. A. (1981) Stimulant-induced psychosis: an Evaluation of animal models. Essays in Neurochemistry and Neuropharmacol., 5 95-129.

Segal, D. S, and Janowsky, D. S. (1978) 'Psychostimulant-induced behavioral effects: possible models of schizophrenia', in Psychopharmacology: A Generation of Progress (Eds. M. A. Lipton, A. DiMascio, and K. F. Killam), Raven Press, New York.

Segal, D. S., Kuczenski, R. (1992a) in vivo microdialysis reveals a diminished amphetamine-induced dopamine response corresponding to behavioral sensitization produced by repeated amphetamine pretreatment. Brain Res., 571 330-337.

Segal, D. S., Kuczenski, R. (1992b) Repeated cocaine administration induced behavioral sensitization and corresponding decreased extracellular dopamine responses in caudate and accumbens. Brain Res., 577 351-355.

Self, D. W. and Nestler, E. J. (1995) Molecular mechanisms of drug reinforcement and addiction. Annual Review of Neurosci., 18 463-496.

Shah, N. S. and Donald, A. G.. (1986) Movement Disorders. Plenum Medical Book Company.

Shimizu, N., Duan, S. M., Hori, T. and Oomura, Y. (1990) Glutamate modulates dopamine release in the striatum as measured by brain microdialysis. Brain Res., 453 353-356.

Simon, H., LeMoal, M., Cardo, B. (1975) Self-stimulation in the dorsal pontine tegmentum in the rat. Behav. Biol., 13 339-347.

Smith, A. D., Bolam, J. P. (1990) The neuronal network of the basal ganglia as revealed by the study of synaptic connections of identified neurons. Trends Neurosci., 13 259-265.

Somers, D. L. and Beckstead, R. M. (1992) N-Methyl-D-aspartate receptor antagonism alters substance P and met5-enkephalin biosynthesis in neurons of the rat striatum, J. Pharmacol. Exp. Ther., 262 823-833.

Spencer, H. J. (1976) Antagonism of cortical excitation of striatal neurons by glutamic acid dyethylester: evidence for glutamic acid as an excitatory transmitter in the rat striatum. Brain Res., 102 91-101.

Starr, M. S. (1987) Opposing roles of dopamine D1 and D2 receptors in nigral [3H] aminobytyric acid release? J. Neurochem., 49 1042-1049.

Stewart, J. And Druhan, J. P. (1993) The development of both conditioning and sensitization of the behavioral activating effects of amphetamine is blocked by the noncompetitive NMDA receptor antagonist, MK-801. Psychopharmacol., 110 125-132.

Stewart, J. And Vezina, P. (1989) Microinjections of SCH-23390 into the ventral tegmental area and substantia nigra pars reticulata attenuate the development of sensitization to the locomotor activating effects of systemic amphetamine. Brain Res., 495 401-406.

Stinus, L., Kelly, A. and Iversen, S. (1978) Increase spontaneous activity following substance P infusion into A10 dopaminergic area. Nature, 276 616-618.

Stoessl, A. J. and Hill, D. R. (1990) Autoradiographic visualization of NK-3 tachykinin binding sites in the rat brain, utilizing [^{3}H] senktid e. Brain Res., 534 1-7.

Suaud-Chagny, M. F., Chergui, K., Chouvet, G. and Gonon, F. (1992) Relationship between dopamine release in the rat nucleus accumbens and the discharge activity of dopaminergic neurons during local in vivo application of amino acids in the ventral tegmental area. Neurosci., 49. 63-72.

Sulzer, D., Maidment, N. T., and Royport, S. (1993) Amphetamine and other weak bases act to promote reverse transport of dopamine in ventral midbrain neurons. J. Neurochem., 60 527-535.

Sulzer, D. and Rayport, S. (1990) Amphetamine and other psychostimulant reduce pH gradients in midbrain dopaminergic neurons and chromaffin granules: A mechanism of action. Neuron, 5 797-808.

Swanson, L. (1982) The projections of the ventral tegmental area and adjacent regions: A combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res. Bull., 9 321-353.

Synder, S. H. (1973) Amphetamine psychosis: a 'model' schizophrenia mediated by catecholamine. Am. J. Psychiatry, 130 61-67.

Taylor, D., and Ho, B. T. (1978) Comparison of inhibition of monoamine uptake by cocaine, methylphenidate and amphetamine. Res. Commun. Chem. Pathol. Pharmacol., 21 67-75.

Thanos, P. K., Jhamandas, K., and Beninger, R. J. (1992) N-methyl-D-aspartate unilaterally injected into the dorsal striatum of rats produces contralateral circling: antagonism by 2-amino-7-phosphonoheptanoic acid and cis-flupenthixol. Brain Res., 589 55-61.

Trifaro, J. M., Vitale, M. L., and Del Castillo, A. R. (1992) Cytoskeleton and molecular mechanisms in neurotransmitter release by neurosecretory cells. Eur. J. Pharmacol., 225 83-104.

Turgeon, S. M., Pollack, A. E., Fink, J. S. (1997) Enhanced CREB phosphorylation and changes in c-Fos and FRA expression in striatum accompany amphetamine sensitization. Brain Res., 749 120-126.

Twery, M. J., Thompson, L. A., and Walters, J. R. (1991) SKF 38393 increases the excitability of striatal neurons ipsilateral to 6-hydroxydopamine-induced dopamine cell lesion and decrease the excitability of neurons in contralateral striatum in vitro. Eur. J. Neurosci., 4 146.

Ujike, H., Tsuchida, H., Kanzaki, A., Akiyama, K., and Otsuki, S. (1992) Competitive and non-competitive N-methyl-d-aspartate antagonists fail to prevent the induction of methamphetamine-induced sensitization. Life Sci., 50 1673-1681.

McCann, U. D., Wong, D. F., Yokoi, F., Villemagne, V., Dannals, R. F. and Ricaurte, G. A. (1998) Reduced striatal dopamine transporter density in abstinent

methamphetamine and methcathinone users: evidence from positron emission tomography studies with [^{11}C]WIN-35,428. J. Neurosci. 18 8417-8422

Ungerstedt, U. (1971) Stereotaxic mapping of the monoamine pathways in the rat brain. Acta. Physiol. Scand., 367 1-48.

Van Dyke, C. and Byck, R. (1982) Cocaine. Sci. Am., 3 128-141.

Van der Kooy, D. (1979) The organization of the thalamic, nigral and raphe cells projecting to the medial vs lateral caudate-putamen in rat. A fluorescent retrograde double labeling study. Brain Res., 169 381-387.

Veening, J. G., Gornelissen, F. M. and Lieven, P. A. J. M. (1980) The topical organization of the afferents of the caudatoputamen of the rat: A horseradish peroxidase study. Neurosci. 5 1253-1268.

Vezina P, Stewart J. (1990) Amphetamine administered into the ventral tegmental area but not to the nucleus accumbens sensitizes rats to systemic morphine: lack of conditioned effects. Brain Res., 516 99-106.

Villemagne, V., Yuan, J., Wong, D. F., Dannals, R. F., Hatzidimitriou, G, Mathews, W. B., Ravert, H. T., Musachio, J., McCann, U. D., Ricaurter, G. A. (1998) Brain dopamine neurotoxicity in baboons treated with doses of methamphetamine comparable to those recreationally abused by humans: evidence from [11C] WIN-35,428 positron emission tomography studies and direct in vitro determinations. J. Neurosci., 18 419-427.

Voorn, P., Roest, G. and Groenewegen, H. J. (1987) Increase of enkephalin and decrease of substance P immunoreactivity in the dorsal and ventral striatum of the rat after midbrain 6-hydroxydopamine lesions. Brain Res., 412 391-396.

Wang, J. (1991) Presynaptic glutamate receptors modulate dopamine release from striatal synaptosomes. J. Neurochem., 57 818-822.

Wang, J. Q. and MeGinty, J. F. (1995) Alternations in striatal zif/268, preprodynorphin and preproenkephalin mRNA expression induced by repeated amphetamine administration in rats. Brain Res., 673 262-274.

Webster, K. E. (1961) Cortico-striate interrelations in the albino rat. J. Anat., 95 532-544.

Weick, B. G. and Walters, J. R. (1988) The D-1 selective agonist SKF 38393 can activate striatal neurons in 6-hydroxydopamine lesioned rats. Sco. Neurosci. Abstr., 14 1077.

Welzl, H., Kuhn, G., huston, J. P. (1989) Self-administration of small amounts of morphine through glass micropipettes into the ventral tegmental area of the rat. Neuropharmacol., 28 1017-1023.

Whitty, C. J., Walker, P. D., Goebel, D. J. and Bannon, M. J. (1993) Cellular localization and regulation of neurokinin receptor gene expression within the substantia nigra. Soc. Neurosci. Abstr., 19 723.

White, F. J., Hu, X. T., Henry, D. J., and Zhang, X. F. (1993) Neurophysiological alterations in the mesocorticolimbic dopamine system with repeated cocaine administration. The Neurobiology of cocaine CRS Chapter 7 99-119.

White, F. J., Hu, X. T., Zhang, X. F., and Wolf, M. E. (1995) Repeated administration of cocaine or amphetamine alters neuronal responses to glutamate in the mesoaccumbens dopamine system. J. Pharmacol. Exp. Ther., 273 445-454.

Willins, D. L., Wallace, L. J., Miller, D. D., and Uretsky, N. J. (1992) a-Amino-3-hydroxy-5-methylisoxazole-4-propionate/kainate receptor antagonist in the nucleus accumbens and ventral pallidum decrease the hypermotility response to psychostimulant drugs. J. Pharmacol. Exp. Ther., 260 1145-1151.

Wilson, C. J., Chang, H. T., and Kitai, S. T. (1982) Origins of postsynaptic potentials evoked in identified rat neostriatal neurons by stimulation in substantia nigra. Exp. Brain Res., 45 157-167.

Wilson, C. J., Chang, H. T., and Kitai, S. T. (1983) Origins of postsynaptic potentials evoked in spiny neostriatal projection neurons by thalamic stimulation in the rat. Exp. Brain Res., 51 217-226.

Winkler, H., Apps, D. K. and Fischer-Colbrie, R. (1986) The molecular function of adrenal chromaffin granules: Established facts and unresolved topics. Neurosci., 18 261-290.

Winkler, H., Sietzen, M., and Schober, M. (1987) The life cycle of catecholamine-storing vesicles. Ann. N.Y. Acad. Sci., 493 3-19.

Wise, R. A. (1980) The dopamine synapse and the notion of "pleasure centers" in the brain. Trends Neurosci., 3 91-94.

Wise, R. A. (1981) Intracranial self-stimulation: mapping against the lateral boundaries of the dopaminergic cells of the substantia nigra. Brain Res., 213 190-194.

Wise, R. and Bozarth, M. (1987) A psychomotor stimulant theory of addiction. Psychol. Rev., 94 469-492.

Wise, R. A., Rompre, I. P. (1989) Brain dopamine and reward. Annu. Rev. Psychol., 40 191-225.

Wise, S. P. and Jones, E. G. (1977) Cells of origin and terminal distribution of descending projections of the rat somatic sensory cortex. J. Comp. Neurol., 175 129-158.

Wolf, M. E. and Khansa, M. R. (1991) Repeated administration of MK-801 produces sensitization to its own locomotor stimulant effects but blocks sensitization to amphetamine. Brain Res., 562 164-168.

Wolf, M. E., White, F. J., Hu, X. T. (1994) MK-801 prevents alterations in the mesoaccumbens dopamine system associated with behavioral sensitization to amphetamine. J. Neurosci., 14 1735-1745.

Yamamoto, B. K., and Davy, S. (1992) Dopaminergic modulation of glutamate release in striatum as measured by microdialysis. J. Neurochem., 58 1736-1742.

Yi, S-J and Johnson, K. M. (1990) Chronic cocaine treatment impairs the regulation of synaptosomal 3H-DA release by D2 autoreceptors. Pharmacol. Biochem. Behav., 36 457-461.

Yi, S-J and Johnson, K. M. (1990) Effects of acute and chronic administration of cocaine on striatal uptake, compartmentalization and release of [3N] dopamine. Neuropharmacol., 29 475.

Yamada, S., Kojima, H., Yokoo, H., Tsutsumi, T., Takamuki, K., Anraku, S., Nishi, S. and Inanaga, K. (1988) Enhancement of dopamine release from striatal slices of rats that were subchronically treated with methamphetamine. Biological Psychiatry, 24 399-408.

Yokel, R. and Wise, R. (1975) Increased lever pressing for amphetamine after pimozide in rats: implications for a dopamine theory of reward. Sci., 187 547-549.

Yoshikawa, T., Shibuya, H., Kaneno, S. and Toru, M. (1991) Blockade of behavioral sensitization to methamphetamine by lesions of the hippocampo-accumbal pathway. Life Sci., 48 1325-1332.

Young, W. S. III, Bonner, T. I. and Brann, M. R. (1986) Mesencephalic dopamine neurons regulate the expression of neuropeptide mRNAs in the rat forebrain. Proc. Natn. Acad. Sci. U.S.A., 83 9827-9831.

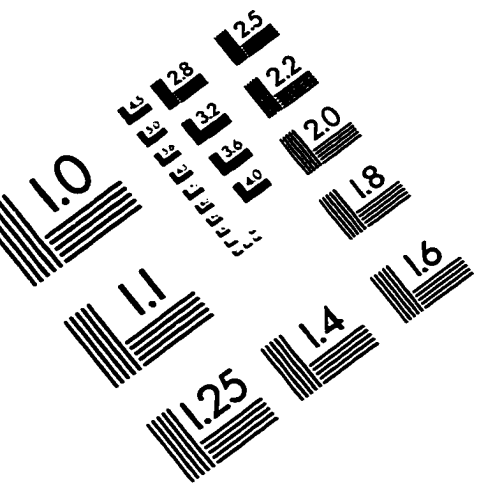
Zhang, F., Van Bree, L., Albala, N., Verslype, M. and Vanderhaeghen, J. J. (1996) NMDA receptor antagonist MK-801 down-regulates rat striatal proenkephalin and protachykinin mRNAs. Neurochem. Int., 28 189-192.

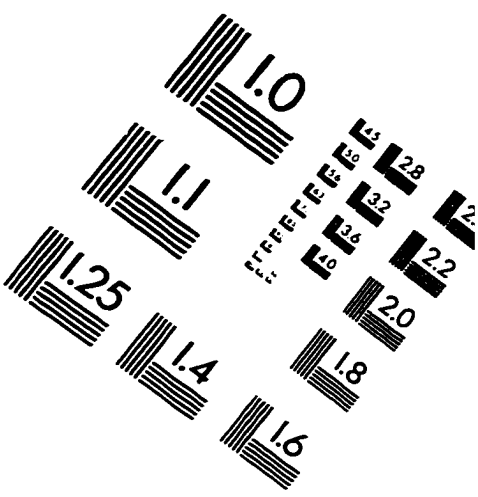
Zhang, H., Lee, T. H., and Ellinwood, E. H., Jr. (1992) The progressive changes of neuronal activities of the nigral dopaminergic neurons upon withdrawal from continuous infusion of cocaine. Brain. Res., 594 315

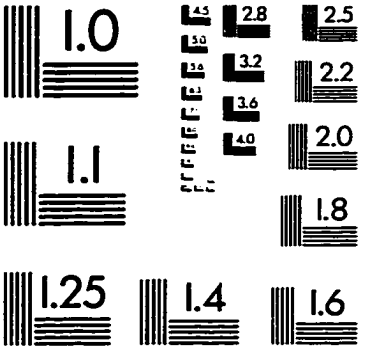
Zhang, J., Chiodo, L. A. and Freeman, A. S. (1992) Electrophysiological effects of mk-801 on rat nigrostriatal and mesoaccumbal dopaminergic neurons. Brain Res., 590 158-163.

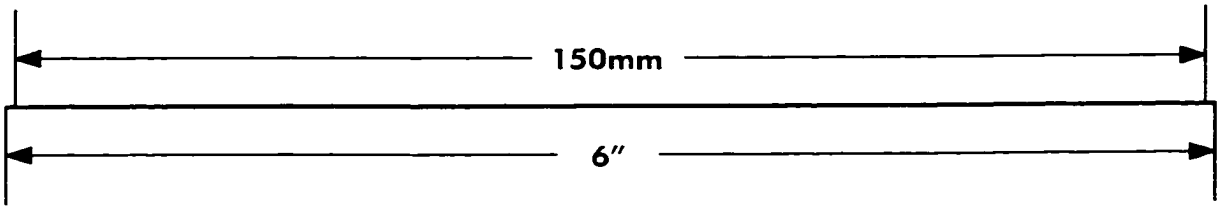
Zhang, Y. and Angulo, J. (1996) Contrasting effects of repetitive treatment versus withdrawal of methamphetamine on tyrosine hydroxylase messenger RNA abundance in ventral tegmental area and substantia nigra zona compacta of the rat brain. Synapse, 24 218-223.

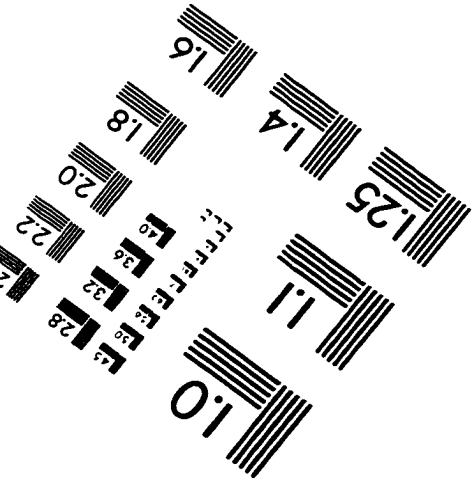
IMAGE EVALUATION TEST TARGET (QA-3)

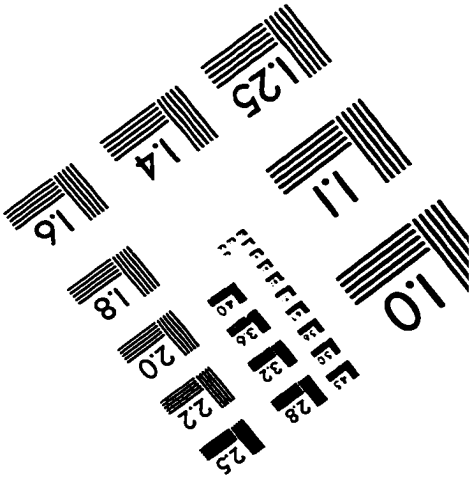












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989