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THE EFFECT OF EARLY PSYCHOSTIMULANT TREATMENT ON ABUSE
LIABILITY AND DOPAMINE RECEPTORS

A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts
in
Psychology:
General-Experimental

by
Steven Wayne Villafranca

December 2005

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Approved by:



Cynthia Crawford, Chair, Psychology

10/26/05
Date



Sanders McDougall



Jean Peacock

ABSTRACT

Given the large number of children treated with methylphenidate, it has become increasingly important to evaluate the possible long-term consequences of methylphenidate treatment. To assess whether the reinforcing properties of drugs of abuse were altered in adulthood, we administered methylphenidate daily from postnatal days 11-20 and then measured preference for morphine during early adulthood using conditioned place preference. The number of dopamine D₂ receptors was measured in each rat and the correlation between receptor number and morphine preference was determined. Regardless of sex or pretreatment group, rats exhibited morphine-induced CPP as rats treated with the 5.0 mg/kg morphine had significantly greater difference scores as compared to rats treated with saline. D₂ binding densities were not altered by methylphenidate pretreatment or morphine exposure. This evidence implies that preweanling rats exposed to methylphenidate can alter drug-rewarded behavior and enhance drug responsiveness in adulthood and suggests that treating young children (e.g. preschool age) with

methylphenidate may increase the risk of drug addiction later in life.

TABLE OF CONTENTS

ABSTRACT	iii
LIST OF FIGURES	vii
CHAPTER ONE: INTRODUCTION	
Overview	1
Proposal	3
CHAPTER TWO: LITERATURE REVIEW	
Dopamine Systems	5
Morphine and Opioid Systems	12
Methylphenidate and Other Psychostimulants	14
Behavioral Effects of Psychostimulants	16
Psychostimulant Neurotoxicity	20
Ontogeny of Dopamine Systems	24
Thesis Statement	28
CHAPTER THREE: METHODS AND MATERIALS	
Subjects	31
Apparatus	31
In Vivo Drug Treatment	32
Morphine-Induced Conditioned Place Preference Procedure	32
Membrane Preparation	34
Homogenate Ligand Binding Assay	34
Statistics	35

CHAPTER FOUR: RESULTS

Body Weight	36
Conditioned Place Preference	38
Locomotor Activity	40
D ₂ Receptor Binding	43

CHAPTER FIVE: DISCUSSION

Overview	47
Effects of Methylphenidate Pre-Exposure on Morphine-Induced Conditioned Place Preference	48
Early Methylphenidate Pre-Exposure and D ₂ Receptors	50
Implications and Conclusions	52

REFERENCES	55
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LIST OF FIGURES

Figure 1. Mean Body Weights During the 10 Days of Methylphenidate Injections. Rats Were Injected With Methylphenidate (0, 2.0, 5.0 mg/kg) from Postnatal Day 11 to Postnatal Day 20 37

Figure 2. Mean Preference Score on Test Day. Rats Were Given Alternating Daily Injections of Morphine (0.0, 0.5, or 5.0 mg/kg) and Saline from Postnatal Day 61 to Postnatal Day 68 39

Figure 3. Mean Locomotor Activity on the First and Last Day of Drug Administration During Conditioned Place Preference Conditioning. During Conditioning, Rats Were Injected with Either 0.0 mg/kg, 0.5 mg/kg or 5.0 mg/kg Morphine 41

Figure 4. Mean Locomotor Activity for Male and Female Rats During Conditioned Place Preference Conditioning 42

Figure 5. Correlation Between Preference Score and Number of Dopamine D₂-Like Binding Sites in Rats Pretreated with Methylphenidate (0, 2.0, or 5.0 mg/kg) from Postnatal Day 11 to Postnatal Day 20 and Conditioned with 5 mg/kg Morphine 44

Figure 6. Correlation Between Preference Score and Number of Dopamine D₂-Like Binding Sites in Rats Pretreated with Saline from Postnatal Day 11 to Postnatal Day 20 and Conditioned with 5 mg/kg Morphine 45

Figure 7. Correlation Between Preference Score and Number of Dopamine D₂-Like Binding Sites in Rats Pretreated with 5.0 mg/kg Methylphenidate from Postnatal Day 11 to

Postnatal Day 20 and Conditioned with 5 mg/kg Morphine	46
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CHAPTER ONE

INTRODUCTION

Overview

In the United States, psychostimulant drugs [e.g., methylphenidate (Ritalin) and amphetamine (Dexedrine; Allderall)] are the most commonly prescribed psychotropic medications given to children (DEA Congressional Testimony, 2000). Psychostimulant treatment is most commonly used for Attention Deficit Hyperactivity Disorder (ADHD), but it is also the preferred treatment for narcolepsy and other disorders (Wilens & Biederman, 1992). It has been estimated that as high as 15% of school-age children meet the DSM-IV diagnostic criteria for ADHD (Scahill & Schwab-Stone, 2000). The large majority of these children are prescribed methylphenidate (Wilens & Biederman, 1992). In fact, the number of children receiving methylphenidate treatment for ADHD has doubled every five years since 1971 (Wilens & Biederman, 1992). Given the large number of children treated with methylphenidate, it has become increasingly important to evaluate the possible long-term consequences of methylphenidate treatment.

While most studies have concluded that methylphenidate has few long-term side effects (Borcherding, Keysor,

Rapoport, Elia, & Amass, 1990; Nolan & Gadow, 1997; Rappley, 1997), more recent research has found that early exposure to methylphenidate can alter later behavior in adulthood. (Andersen et al., 2002; Crawford et al., 2000). For instance, exposing adolescent rodents to methylphenidate causes an increase in cocaine self-administration and cocaine-induced conditioned place preference (Achat-Mendes et al, Brandon, Marinelli, Baker, & White, 2001). Moreover, similar results have been demonstrated in humans, as a recent clinical study found that college students were more likely to abuse cocaine if they had previously received methylphenidate during childhood or adolescence (Schenk & Davidson, 1998).

While the cause of this change in responsivity to cocaine is unknown, it is possible that changes in dopamine receptors may be involved. Repeated treatment with the psychostimulant methamphetamine during the preweanling period has resulted in a long-term reduction in the number of dopamine D₂ receptors (Crawford et al., 2000a). Moreover, recent imaging studies in humans indicate a relationship between drug addiction and number of dopamine D₂ receptors (Volkow, Fowler, Wang, & Goldstein, 2002). Specifically, the number of dopamine D₂ receptors are predictive of the rewarding properties of psychostimulants. Subjects with

higher numbers of dopamine receptors did not find acute treatment with a psychostimulant pleasurable, while subjects with lower numbers of receptor sites did find the drug treatment pleasurable. Since methylphenidate and methamphetamine share a similar mechanism of action (Fumagalli, Gainetdinov, Wang, Valenzano, Miller, & Caron, 1999), it is possible that early methylphenidate treatment may decrease dopamine binding sites and thus increase the probability of later drug abuse.

Proposal

It now appears that early methylphenidate treatment may have long-term effects on behavior. In particular, early methylphenidate treatment may increase later vulnerability to drugs of abuse. In order to assess whether the reinforcing properties of drugs of abuse are altered in adulthood, we will administer methylphenidate daily from postnatal day (PD) 11-20 and then measure preference for morphine during early adulthood (i.e., PD 60) using conditioned place preference. In addition, rats in this study will be sacrificed at the end of preference conditioning and their dorsal striata removed. The number of dopamine D₂ receptors will be measured in each rat and

the correlation between receptor number and morphine preference will be determined.

CHAPTER TWO

LITERATURE REVIEW

Dopamine Systems

Dopamine is an important catecholamine neurotransmitter involved in the mediation of reward, motor behavior, and memory. Dopamine synthesis begins with the amino acid tyrosine, which is converted to L-dihydroxyphenylalanine (L-DOPA) in the presence of the enzyme tyrosine hydroxylase (Siegel, Agranoff, Albers, Fisher, & Uhler, 1999). L-DOPA is then converted to dopamine by the enzyme DOPA decarboxylase (or aromatic amino acid decarboxylase) (Siegel et al., 1999). Conversion of tyrosine to L-DOPA, as well as L-DOPA to DA occurs in the cytosol (Elsworth & Roth, 1997). Free dopamine is then pumped into vesicles by the vesicular monoamine transporter (VMAT). Dopamine is released by calcium-dependent exocytosis (Robitaille, Adler, & Charlton, 1990). During this process, an action potential at the axon terminal triggers the opening of voltage-gated calcium channels inducing calcium ion influx and vesicular release of dopamine (Robitaille, Adler, & Charlton, 1990). Newly synthesized dopamine is preferentially released, although stored dopamine can be mobilized after repeated stimulation. After release, dopamine is primarily

adenylyl cyclase activity, D₂ receptors actually inhibit adenylyl cyclase activity (Baldessarini & Tarazi, 1996). As more receptor subtypes were identified over the years (D₁ through D₅), Clark and White (1987) classified the receptor subtypes into D₁-like (D₁ and D₅) and D₂-like (D₂, D₃, and D₄) families. As research continued, two distinct forms of the D₂ receptor became evident; D_{2(long)} and D_{2(short)}. These D₂ receptor subtypes were found to differ by only a 29-amino acid insertion in the intracellular loop (Smith, Fetsko, & Wang, 2002). Clark and White (1987) devised their nomenclature through an extensive review of studies using both dopamine agonists and antagonists.

Dopamine agonists, which compete for the same receptor, cause the same effect as the endogenous neurotransmitter and stimulates the receptor. Typical D₁/D₅ agonists include SKF 38393, fenoldopam, and dihydrexidine (Gorelova & Yang, 2000). Although no compounds differentiate D₁ and D₅ receptors, these receptors differ in other aspects. The most widely accepted difference between D₁ and D₅ receptors is that D₅ receptors display a higher affinity for dopamine than do D₁ receptors (Grandy et al., 1991; Sunahara et al., 1991; Tiberi et al., 1991). This observation is in accord with later research showing that D₅ receptors have a stronger coupling to G-proteins than do D₁ receptors (Kimura

et al., 1995). Quinpirole and pergolide represent D₂ and D₃ selective agonists (Feldman, Meyer, & Quenzer, 1997). Quinpirole, however, is more selective for D₂ receptors than D₃ receptors. Conversely, pramipexole, binds with a higher affinity to D₃ receptors than D₂ receptors (Bennett & Piercey, 1999). Common D₄ agonists consist of CP-226 and CP-269 (Oak, Oldenhof, & Van Tol, 2000).

Antagonists also bind to receptor sites, however, they do not activate the receptor. Instead, antagonists prevent the endogenous neurotransmitter, and other molecules, from binding to and stimulating the receptor (Watkins, Krosgaard-Larsen, & Honore, 1990). Typical D₁/D₅ receptor antagonists include SCH 23390, NNC-112, and SCH 39166 (Gorelova & Yang, 2000). Selective D₂ receptor antagonists include haloperidol, sulpiride, spiperone, and YM-09151-2. Nafadotride and PD 152255 are newly developed D₃ receptor antagonists (Sobrian, Jones, Varghese, & Holson, 2003). Clozapine is representative of a D₄ receptor antagonist (Van Tol et al., 1991).

Both D₁-like and D₂-like dopamine receptors are guanosine triphosphate protein (G-protein) coupled receptors. As previously mentioned, D₂-like receptors inhibit adenylyl cyclase activity. This inhibition is due to the fact that D₂-like receptors are coupled to inhibitory

G-proteins (G_i). Conversely, D_1 -like receptors are coupled to stimulating G-proteins (G_s , G_{olf}), and increase adenylyl cyclase activity (Baldessarini & Tarazi, 1996; Hervé et al., 2001). When activated, G_s proteins stimulate adenylyl cyclase, which converts adenosine triphosphate (ATP) into cyclic adenosine monophosphate, or cAMP (Baldessarini & Tarazi, 1996; Hervé et al., 2001). In turn, cAMP activates protein kinase A (PKA), which effects both calcium and potassium permeability (Siegel et al., 1999).

The highest concentration of D_1 receptors are found in the caudate, putamen, nucleus accumbens, olfactory tubercles, and substantia nigra (Tarazi, Tomosine & Baldessarini, 1999). Low concentrations of D_1 receptors also reside in the neocortex, thalamus, cerebellum, and septum (Wamsley, Alburges, McQuade, & Hunt, 1992). D_5 receptors, in contrast, are essentially limited to the hippocampus and hypothalamus (Tarazi, Tomosine & Baldessarini, 1999). D_2 -like receptors have a pattern similar to D_1 -like receptors. For example, D_2 and D_3 receptors are both found in high concentrations in the olfactory tubercles and the nucleus accumbens. In addition, D_3 receptors are found in the islands of Calleja. D_4 receptors are expressed in small quantities in the striatum (Van Tol et al., 1991), but are fairly prevalent in

the retina (Cohen, Todd, Harmon, & O'Malley, 1992), cerebral cortex, amygdala, hypothalamus, and the pituitary (Asghari, Sanyal, Buchwaldt, Paterson, Jovanovic, & Van Tol, 1995; Valerio, Belloni, Gorno, Tinti, Memo, & Spano, 1994).

Dopamine Pathways

Dopamine is found in two major pathways in the central nervous system: the nigrostriatal and the mesolimbic. In the mesolimbic system, dopamine is produced by neurons in the ventral tegmental area (VTA), which project to structures such as the prefrontal cortex and the basal forebrain, including the nucleus accumbens (McBride, Murphy, & Ikemoto, 1999; Wise & Bozarth, 1984). The mesolimbic system is thought to be associated with reward (Bozarth, 1991). The mesolimbic system is stimulated by natural means; such as nutriment, predation, and intercourse, as well as artificially by psychostimulants (Di Chiara, 1999; Leshner & Koob, 1999; Wise & Bozarth, 1984).

Olds and Milner (1954) provided preliminary empirical evidence regarding reward and reinforcement in the mesolimbic system. These researchers electrically stimulated the medial forebrain bundle (MFB), and found that it was rewarding to subjects, as evidenced by repeat bar pressing. Additionally, lesioning the MFB disrupts reward mediated behavior (Wise & Bozarth, 1987). Due to the fact

that dopaminergic fibers terminate in the nucleus accumbens, Bozarth (1991) posits that the nucleus accumbens is the central location of the rewarding action of drugs of abuse. Researchers have since found that the activation of the mesolimbic system results in increased locomotor activity (Wise & Bozarth, 1987).

The second major dopamine pathway, termed the nigrostriatal pathway, extends from the substantia nigra to the dorsal striatum; which contains the caudate and the putamen (White, 1996). Dopamine concentrations are at their highest in the caudate and putamen (Jaber, Robinson, Missale, & Caron, 1996). The striatum is an important motor center, as dysfunctions in striatal dopamine have been found to cause motor disorders such as Parkinson's disease and Tourette's syndrome (Bennett & Piercey, 1999; Kurlan, Behr, Medved, & Como, 1988). The dorsal striatum is also implicated in habit learning such as learning to automatically perform complex motor tasks, such as driving an automobile (Di Chiara, 1990). Moreover, the striatum is now believed to be a component of a memory circuit associated with craving (Volkow et al., 2002) and the formation of habits associated with chronic drug use (White, 1996).

Morphine and Opioid Systems

Opioids are drugs that are widely used for their analgesic properties, but are also among the most abused drugs. The opioid compounds work by activating a group of g-protein coupled receptors. Three types of opioid receptors have been discovered: mu, delta, and kappa. All the opioid receptors are coupled to the inhibitory G-protein, $G_{i/o}$, which inhibits adenylyl cyclase activity and can directly open K^+ and Ca^{++} channels (Waldhoer, Bartlett, & Whistler, 2004). Opioid receptors are distributed throughout the central nervous system, however, the nucleus accumbens, caudate putamen, and hypothalamus are particularly abundant in mu and delta receptors; while kappa receptors are primarily found in the hypothalamus, small quantities are also found in the caudate putamen and nucleus accumbens (Unterwald, Rubenfeld, Imai, Wang, Uhl, & Kreek, 1995; Spangler, Ho, Zhou, Maggos, Yuferov, & Kreek, 1996).

There are four classes of endogenous opioid ligands, derived from precursor molecules via enzymatic processing, which activate mu, delta, or kappa receptors (Hertz, 1997). Beta endorphin is derived from the precursor pro-opiomelanocortin and displays a high affinity for mu receptors and low affinity for delta receptors. Proenkephalin gives rise to two types of enkephalins:

methionine and leucine. Enkephalins exhibit some selectivity for delta receptors, while dynorphins show some selectivity for kappa receptors. Recent research has identified a fourth class of endogenous opioid ligand; endomorphin 1 and 2, which exhibits an extremely high selectivity for mu receptors (Zadina, Hackler, Ge, & Kastin, 1997).

The opioid system, like the dopamine system, is an important part of the neural reward circuitry. The reinforcing actions of opiates are largely mediated by the mu opioid receptor subtype. Much like psychostimulants, mu opioid agonists, such as morphine are readily self-administered by animals and induce conditioned place preferences. A large number of mu opioid receptors reside in the VTA and nucleus accumbens and when activated increase the release of dopamine (Di Chiara & North, 1992; Koob, 1992). This mu opioid-induced release of dopamine is believed to mediate the reinforcing properties of opiates. However, while some of the rewarding aspects of mu agonists are dopamine dependent other research has indicated that mu agonists also use dopamine independent pathways (Di Chiara & North, 1992; Koob, 1992). Specially, these studies have demonstrated that self-administration of morphine is not blocked by D₂ antagonists. However, other studies suggest

that some dopamine antagonists (i.e. D₁) can block, or partially block (i.e. 6-OHDA), morphine administration (Shippenberg & Herz, 1987; Shippenberg & Herz, 1988; Acquas, Carboni, Leone, & Di Chiara, 1989; De Fonseca, Rubio, Martin-Calderon, Caine, Koob, & Navarro, 1995).

Methylphenidate and Other Psychostimulants

Overview

Psychostimulants are heterogeneous groups of compounds that share the ability to increase arousal and activity levels by activating the sympathetic nervous system, and increasing levels of extracellular monoamines in the central nervous system (Creese, 1982). These compounds have been medically utilized for their stimulant properties for many years (Creese, 1982). For example, psychostimulants are used to increase attention levels in ADHD and to increase arousal levels in narcolepsy (Solomon, White, Parron, & Mendelson, 1979). In addition to these neuropsychiatric disorders, psychostimulants are used to treat obesity as they increase body metabolism and suppress appetite (Martin, Sloan, Sapira, & Jasinski, 1971; Silverstone, 1981).

Unfortunately, the stimulant properties of these drugs are also exploited recreationally as psychostimulants are among

the most addictive and abused class of drugs (Fischman & Haney, 1999).

Mechanisms of Action

Cocaine and amphetamine are prototypical psychostimulants that increase synaptic levels of monoamine neurotransmitters (Creese, 1982). Cocaine increases synaptic levels of monoamines by inhibiting the actions of dopamine, norepinephrine, and serotonin transporter sites; however, the reuptake inhibiting actions of cocaine are much more prominent at dopamine and serotonin synapses (Deutsch & Schweri, 1994; Kuczenski & Segal, 1997; Pan et al., 1994; Schenk & Izenwasser, 2002). Amphetamine also increases dopamine levels, as well as extracellular levels of norepinephrine and serotonin (Kuczenski & Segal, 1997). Compared to cocaine, amphetamine has a more complicated mechanism of action as it increases monoamine levels by reversing the direction of the monoamine reuptake pumps, blocking reuptake, and by acting as a monoamine oxidase inhibitor (Yokel & Wise, 1975). Amphetamine's ability to reverse monoamine transporters to release newly synthesized dopamine and norepinephrine appears to be the most important of its pharmacological actions (Fumagalli, Gainetdinov, Wang, Valenzano, Miller, & Caron, 1999). This releasing action leaves vast quantities of dopamine and norepinephrine

free in the synaptic cleft, and produces the 'rush' reported by drug users (NIDA, 1999).

The psychostimulant, methylphenidate, is structurally related to amphetamine but has a mechanism of action similar to cocaine. That is to say, methylphenidate increases monoamine levels in the synapse primarily by blocking transporter sites. However, methylphenidate, unlike cocaine, has a very low affinity for the serotonin transporter, and, consequently, is a poor in vitro inhibitor of serotonin uptake (Pan et al., 1994; Schenk & Izenwasser, 2002). Methylphenidate also appears to increase the release of monoamines using a mechanism similar to amphetamine; through interaction with monoamine transporters.

Behavioral Effects of Psychostimulants

Acute Effects in Animals

Psychostimulant administration produces a wide range of behavioral actions. In rodents, acute psychostimulant exposure primarily induces locomotor activity at low to moderate doses while producing intense oral stereotypies at higher doses (Kelly & Iversen, 1976; Taylor & Snyder, 1971; Tirelli, Laviola, & Adriani, 2003; Yang, Amini, Swann, & Dafny, 2003). For example, acute injections of low doses of methylphenidate (under 2.0 mg/kg) produce no significant

effects on locomotor activity (Yang et al., 2003), while acute injections of methylphenidate at high doses (2.5 to 40 mg/kg) produce immediate locomotor effects (Yang et al., 2003). Similarly, low doses of cocaine, and particularly amphetamine, cause an increase in locomotor activity, while higher doses of both psychostimulants induce pronounced stereotyped behavior (Kelly & Iversen, 1976).

Acute Effects in Humans

Low doses of acute amphetamine in humans results in feelings of well-being, increased competence and alertness, as well as reduced appetite (NIDA, 1998). Higher doses of acute amphetamine can cause tremors, sweating, and heart palpitations in humans (NIDA, 1998). Low doses of acute cocaine in humans results in a variety of subjective effects including: euphoria, talkativeness, increased energy and mental alertness (NIDA, 1999). Low doses of acute cocaine in humans also produces physiological effects such as: dilated pupils, increased temperature, heartrate, and blood pressure (NIDA, 1999). High doses of acute cocaine results in bizarre, erratic, and violent behavior (NIDA, 1999). Acute administration of low doses of methylphenidate in humans results in elevated blood pressure, tachycardia, increased respiration, suppressed appetite, and sleep depravation (NIDA, 2001). Acute administration of high

doses of methylphenidate can cause delirium, aggressiveness, panic states, and hallucinations (Morton & Stockton, 2000).

Chronic and Repeated Effects

In rodents, repeated intermittent exposure to cocaine and amphetamine result in a phenomenon called reverse tolerance or behavioral sensitization (Kalivas & Stewart, 1991). This phenomenon is characterized by a progressive and enduring enhancement of the drug-induced behavioral effects of psychostimulant compounds (Kalivas & Stewart, 1991; Robinson & Berridge, 2001; Sorg & Newlin, 2002). Behavioral sensitization can be observed after as little as one drug exposure and can still be detected for at least a year after the last drug exposure (Kalivas & Stewart, 1991; Robinson & Becker, 1986). Much of the interest generated by behavioral sensitization stems from its utility as an animal analog of human psychosis (Kalivas & Stewart, 1991) and as a model of human drug addiction (Davidson, Gow, Lee, & Ellinwood, 2001). Repeated injections of methylphenidate can also produce behavioral sensitization, with exponential dosage increases resulting in tolerance (Yang et al., 2003).

Marked neurotoxic effects can occur during chronic and repeated psychostimulant dosing schedules (Davidson, Gow, Lee, & Ellinwood, 2001). For example, exposure to high

doses of methamphetamine causes long lasting reductions in dopamine content, tyrosine hydroxylase activity, decreased dopamine release, and reductions in the number of dopamine transporters (Bowyer et al., 1992; Eisch, Gaffney, Weihmuller, O'Dell, & Marshall, 1992; Kokoshka, Fleckenstein, Wilkins, & Hanson, 2000; O'Dell, Weihmuller, & Marshall, 1993; Ricaurte, Schuster, & Seiden, 1980; Sabol, Roach, Broom, Ferreira, & Preau, 2001; Wagner, Ricaurte, Johanson, Schuster, & Seiden, 1980).

In humans, repeated amphetamine administration produces paranoia, delusions, hallucinations, and violent behavior (NIDA, 1998). Chronic administration of cocaine in humans also produces several changes in behavior including: irritability, mood disturbances, restlessness, paranoia, and auditory hallucinations (NIDA, 1999). Chronic administration of methylphenidate can result in psychotic symptoms similar to amphetamine (Morton & Stockton, 2000).

Abuse Potential

Both amphetamine and cocaine are highly addictive and widely abused drugs (Kollins, MacDonald, & Rush, 2001). The National Household Survey on Drug Abuse (2000) estimates that 4.0% of the population have tried methamphetamine at some point in their lives. The National Institute on Drug Abuse (1999) reports that 1.5 million Americans age 12 and

older are chronic cocaine users, and 0.9 million abuse methylphenidate. Increases in the recreational usage of methylphenidate has raised concerns regarding its abuse potential (Johnston, O'Malley, & Bachman, 2003; DEA Congressional Testimony, 2000). The illicit use and abuse of methylphenidate is well known, and has been documented throughout recent decades. The illicit use of methylphenidate among high school seniors increased from 0.1% in 1992 to 2.8% in 1997 (Johnston, O'Malley, & Bachman, 2003). Methylphenidate is abused orally (Lucas and Weiss, 1971), intranasally (Jaffe, 1991), and through intravenous administration (Levine, Caplan, & Kauffman, 1986). Volkow and colleagues (1995) found that when methylphenidate is abused intranasally, the effects are similar to the intranasal use of amphetamine.

Psychostimulant Neurotoxicity

One proposed mechanism for psychostimulant-induced toxicity is the production of free radicals. A free radical is a molecular species with an unpaired electron. High-dose administration of amphetamine and its analogues increase free radicals (Colado et al., 1997; Fleckenstein et al., 1997; Giovanni, Liang, Hastings, & Zigmond, 1995). Molecular oxygen is then released via enzyme degradation and

through non-enzymatic means (auto-oxidation) (Davidson, Gow, Lee, & Ellinwood, 2001). The enzyme MAO metabolizes dopamine to DOPAC and hydrogen peroxide. The resulting oxygen molecules become highly reactive with other molecules, hence, they are termed reactive oxygen species (ROS). ROS eventually reacts with hydrogen peroxide forming a hydroxyl free radical (Feldman et al., 1997). These free radicals are actually produced during the normal functioning of cells, however, they are kept in balance by antioxidants. In the case of a hydroxyl free radical, there is no existing enzyme reactive enough to remove it (Feldman et al., 1997). This is dangerous because necrotic cell death can occur (Davidson et al., 2001).

Necrotic cell death is induced by a chain reaction that occurs from the reaction between free radicals and a non-radical compound. Necrosis does not affect individual cells, but is marked by swelling and inflammation of the cell in general, as well as the mitochondria (Brown & Yamamoto, 2003). Methamphetamine specifically has the ability to disrupt the electrical gradient of mitochondrial plasma membranes. This eventually results in the cells contents being ousted, and the cell dying (Davidson et al., 2001). In the case of methamphetamine administration, necrosis may be favored over apoptosis due to ROS formation

and mitochondrial inhibition (Brown & Yamamoto, 2003).

Apoptotic cell death is an irreversible mechanical injury to the cell. Apoptosis affects individual cells and is characterized by cell shrinkage and fragmentation. The pattern of events in apoptosis is so orderly that the process is often called programmed cell death. The process of apoptosis is intimately intertwined with mitochondria. Mitochondria produces adenosine triphosphate (ATP), which is the energy the cell utilizes to carry out its various functions. The activation of apoptosis requires ATP. Hyperthermia increases the utilization of cellular ATP, providing a potential basis for amphetamines induction of necrosis over apoptosis (Madl & Allen, 1995). The absence or presence of ATP depletion, in conjunction with the inhibition of mitochondrial function, may play a role in determining which type of cell death is induced (Leist, Single, Castoldi, Kuhnle, & Nicotera, 1997; Qian, Herman, & Lemasters 1999). The mechanisms involved in the switch from apoptosis to necrosis are not fully understood, but changes within the mitochondria are indicated (Brown & Yamamoto, 2003). Dopaminergic neurons have a very high energy demand, making them particularly sensitive to mitochondrial damage (Davidson et al., 2001). Both apoptotic and necrotic cell death appear to contribute to psychostimulant neurotoxicity.

Due to the massive influx of dopamine into the extracellular space upon psychostimulant introduction, the second consideration of neurotoxic effects must necessarily involve neurotransmitter levels. Excessive monoamine levels, due to psychostimulant exposure, inevitably lead to the activation of apoptotic and necrotic mechanisms. Besides an obvious reduction in dopamine stores, a downregulation of D₂-like receptors is also likely to occur. This takes place because the remaining receptors must now compensate for the receptors that are no longer functioning. As dopaminergic receptors are killed off by the neurotoxic effects of the drugs, the system becomes downregulated, and the surviving receptors often become sensitized due to their now reduced numbers (Nader et al., in press). The inverse of this effect is the increase of receptors, known as upregulation. The increase in the numbers of receptors is often accompanied by desensitization. It has been widely noted that the neurotoxicity caused by the various amphetamine analogs results in long-term reductions in dopamine content (Clausing et al., 1995; Fields, Wichlinski, Drucker, Engh, & Gordon, 1995). On the other hand, other psychostimulants, such as cocaine, have failed to yield long-term dopaminergic deficits (Capon et al., 1997; Kleven, Perry, Woolverton, & Seiden, 1990; Yeh & De Souza, 1991;).

Ontogeny of Dopamine Systems

Ontogeny of Dopamine Receptors

Determining the precise ontogeny of D₁ and D₂ receptors has proved difficult as conflicting results of the development of dopamine receptors have been reported. However, it is clear that dopamine receptors are present very early in development as both striatal D₂ mRNA and D₂ receptor binding have been detected prenatally in rats (De Vries, Mulder, Schoffelmeer, 1992; Schambra, Duncan, Breese, Fornaretto, Caron, & Freneau, 1994). D₁ mRNA has also been detected as early as gestational day 11, but the receptors are not detectable prenatally (Broaddus & Bennet, 1990; Cadoret, Jaber & Bloch, 1993). Development of both D₁ and D₂ dopamine receptors appears to be complete by the third or fourth postnatal week (Broaddus & Bennett, 1989; De Vries et al., 1992; Schambra et al., 1994). D₁ receptors, however, appear to reach adult levels faster than D₂ receptors (Broaddus & Bennett, 1989; Rao, Molinoff, & Joyce, 1991; Schambra et al., 1994). During the periadolescent period (PND 34-46) striatal D₁ and D₂ receptors actually increase above adult levels and are pruned down once adulthood is reached (Andersen, 2003; Teicher, Krenzel, Thompson, & Andersen, 2003).

Ontogeny of Psychostimulant-Induced Behavior

Psychostimulants have a multitude of effects in the developing CNS. These effects are usually similar to those found in adults, however, ontogenetic differences do exist. For instance, acute injections of psychostimulants increase activity in both adults and pups, while chronic or repeated dosing schedules produce differential effects (Kalivas & Stewart, 1991; Robinson & Berridge, 2001). Specifically, repeated exposure to psychostimulants causes a progressive and long-lasting increase in psychomotor activating effects, a phenomenon which is referred to as behavioral sensitization (Robinson & Becker, 1986). In adult rats, repeated exposure to psychostimulants in general results in sensitization that can be detected long (i.e., up to a year) after the cessation of drug administration (Robinson & Becker, 1986). In contrast, behavioral sensitization does not occur until the first week postnatally, moreover, expression of the sensitized response is transient upon the cessation of drug administration. For example, when either methylphenidate or amphetamine was administered for 4 or 5 days to rats at PD 10-11 or 16-17, sensitization was exhibited when tested 1 or 2 days after the cessation of the dosing regimen, but not 1 week later (McDougall, Collins, Karper, Watson, & Crawford, 1999; McDougall, Duke, Bolanos,

& Crawford, 1994). The long-term retention of behavioral sensitization matures progressively and is not complete until the third postnatal week (Tirelli, Laviola, & Adriani, 2003).

Ontogeny of Psychostimulant Toxicity

High doses of methamphetamine alter dopaminergic neurons in the adult rat (Davidson et al., 2001). Numerous research studies have shown that high doses of methamphetamine cause long-term changes in dopamine content, tyrosine hydroxylase activity, decreased dopamine release, and reduced numbers of dopamine transporters (Bowyer et al., 1992; Eisch et al., 1992; Kokoshka et al., 2000; Ricaurte et al., 1980; O'Dell et al., 1993; Sabol et al., 2001; Wagner et al., 1980). However, early postnatal exposure to amphetamine and its analogs have less of an effect on dopamine levels in comparison with exposure in adults (Lucot, Wagner, Schuster, & Seiden, 1982; Miller, O'Callaghan, & Ali, 2000; Wagner et al., 1980). While the cause of this differential response to amphetamine administration is unknown, it is possible that it is due to an underdeveloped dopaminergic system in rat pups. For example, a study comparing the dopaminergic reuptake system in 5-day old rat pups and adult rats found that the rat pups were less efficient at removing dopamine from the

extracellular environment as compared to adult rats (Gazzara & Andersen, 1997). This difference in the amount of dopamine removed is explained by fewer uptake sites in the pups due to immature nerve terminals (Coulter, Happe, & Murrin, 1996; Voorn, Kalsbeek, Jorritsma-Byham, & Groenewegen, 1988).

In contrast to the inability of amphetamine and derivatives to cause changes in dopaminergic markers in young animals, several studies have indicated that early psychostimulants do have long-term effects when assessed in adulthood. For instance it has been found that neonatal exposure to methamphetamine causes learning impairments on cognitive tests in adult rats (Vorhees, Ahrens, Acuff-Smith, Schilling, & Fisher, 1994; Vorhees, Inman-Wood, Morford, Broening, Fukumura, & Moran, 2000). More specifically, deficits in spatial learning and memory were found after neonatal methamphetamine treatment (Vorhees et al., 1994; 2000). This research indicates that the neonatal period, up to at least PD 20, is extremely sensitive to the effects of a wide range of psychostimulants. In addition to the long-term effects on learning and memory, a recent study has found that preweanling rats exposed to methamphetamine exhibited a reduction in dorsal striatal D₂-like binding sites and dopamine levels that were still detectable into adulthood (Crawford et al., 2003).

Thesis Statement

The most commonly diagnosed childhood behavioral disorder is ADHD, which is widely treated with the psychostimulant methylphenidate (Swanson, Seargeant, Taylor, Sonuga-Barke, Jensen, & Cantwell, 1998). Recent studies have indicated that early methylphenidate exposure may have long lasting effects, especially on drug responsivity in adult rats and humans. Thus, the purpose of this research project is to determine whether chronic treatment with methylphenidate during the preweanling period will alter the reinforcing properties of morphine in young adult rats. To determine whether morphine is reinforcing, the condition place preference (CPP) paradigm was used. CPP has been used extensively to measure drug reward (Bardo, Rowlett, & Harris, 1995). The basic premise of CPP holds that when a drug (US) is paired with contextual cues (CS), the environmental stimuli acquires the properties of the US. In the CPP paradigm, a drug is repeatedly administered in the same distinctive environment, resulting in the animal associating the drug effect with the environment. Each chamber within the CPP apparatus differs in the visual, somatosensory, and olfactory cues, which provides distinct environments (Stolerman, 1992). Following the appropriate number of conditioning trials, the experimental animals are

allowed to choose freely between the drug-paired and the drug-free chamber. Time spent in the drug-paired chamber, relative to the drug-free chamber, is indicative of place preference, and is considered a behavioral measure of a drug's reinforcing effect. In the majority of CPP experiments, the vehicle (typically saline) and the drug are paired with different compartmental environments (Bardo et al., 1995). An increase in the amount of time spent in a chamber that had previously been paired with a drug is believed to be analogous to drug seeking behavior in human subjects. CPP is now believed to model the motivation elicited by environmental stimuli involved in drug-taking behavior (Bardo et al., 1995).

After preference for the morphine- and saline-paired compartments are determined, the rats were sacrificed and the number of dopamine D₂ receptors measured. I predict that rats given early methylphenidate exposure will (a) spend a greater amount of time in the morphine-paired environment than saline-exposed rats and (b) preference for the drug-paired compartment will be negatively correlated with the number of dopamine D₂ receptors.

If the results showed that early methylphenidate exposure increases drug preference and decreases dopamine receptors; it supports the hypothesis that there is a

biological reason for increased drug use after methylphenidate treatment. If the results showed that early methylphenidate treatment does not change drug preference or dopamine receptors, this supports the hypothesis that early methylphenidate treatment does not increase the probability of later drug use.

CHAPTER THREE
METHODS AND MATERIALS

Subjects

Subjects were 108 male and female rats of Sprague-Dawley descent (Harlan), born and raised at California State University, San Bernardino. Litters were culled to ten pups by 3 days of age, and kept with the dam throughout behavioral testing. Pups were kept with the dam until PND 25, at which time they were weaned and placed in group cages with same-sex litter mates. Only one rat from each litter was placed into a particular group. The colony room was maintained at 22-24°C and kept under a 12-hr light/dark cycle. Behavioral testing was done during the light cycle, at approximately the same time each day. Subjects were treated according to the American Psychological Associations "Ethical Principles" (1992), and the Principles of Laboratory Animal Care (National Institute of Health Publication # 85-23).

Apparatus

CPP was done in T-shaped wooden chambers comprised of three compartments. The two large end compartments (24 × 30

x 45 cm) are adjacent to each other and are separated by a removable partition. The small compartment (the placement chamber, 24 x 10 x 45 cm) projects out from the junction between the large compartments. A second removable partition enables rats to enter either of the large compartments from the placement chamber. The odor, flooring, and color of each compartment vary. One of the large end compartments has white walls, wire mesh flooring, and pine bedding, whereas the other large end compartment has black walls, metal rod flooring, and cedar bedding. The placement chamber has a solid wood floor and is painted gray.

In Vivo Drug Treatment

Starting at PND 10, rats received daily injections of methylphenidate (0.0, 2.0, or 5.0 mg/kg, ip). These daily injections continued for 10 consecutive days.

Morphine-Induced Conditioned Place Preference Procedure

Following acclimation to handling, a total of 108 sixty-day-old rats from the three pretreatment conditions were randomly assigned to groups. A 10-day biased CPP procedure was used, which included one preconditioning day,

eight conditioning days (consisting of alternating daily injections of saline or morphine) and one test day. On the preconditioning day, rats received no injection and were put in the gray placement chamber of the apparatus. After rats entered either the black or white conditioning compartment, access to the placement chamber was blocked and rats were allowed 15 minutes access to the black and white compartments. On the conditioning days, rats either received an injection of morphine (0, 2, or 5 mg/kg, sc) and were placed in their non-preferred compartment or they received an injection of saline and were placed in their preferred compartment. Initial drug order was counterbalanced between groups. Conditioning sessions lasted 30 minutes. On the test day, rats were left uninjected and given free access to the black and white compartments for 15 minutes.

All conditioning and test days were videotaped and time spent in the non-preferred compartment and locomotor activity was scored by experimenters blind to treatment conditions. Locomotor activity was assessed by dividing the two end compartments into four equal quadrants and counting the number of times each rat crosses into a different quadrant. In summary, a 2 x 3 x 3 (sex x pretreatment

condition × drug group) experimental design was used. There was a total of six rats per group.

Membrane Preparation

Immediately following behavioral testing, rats were sacrificed and their dorsal striata removed. The tissue was stored at -80° C until time of assay. On the assay day, tissue was thawed on ice and crude membrane homogenates was made using the following protocol. Striatal sections from each rat were homogenized in 100 volumes of 50 mM Tris-HCl buffer (pH 7.4) for approximately 20 s using a Brinkmann Polytron. The homogenates were centrifuged at $20,000 \times g$ for 20 minutes. The pellet was resuspended in 100 volumes of the same buffer and centrifuged again at $20,000 \times g$ for 20 minutes. The final pellet was suspended in approximately 30 volumes of buffer (pH 7.4). Protein concentrations for the final pellet were determined using the Bio-Rad Protein Assay with BSA as the standard.

Homogenate Ligand Binding Assay

For the D_2 receptor binding assay, tissue suspensions (50-100 μ g/protein) were added to duplicate tubes containing 50 mM Tris, 2 mM $NaCl_2$, 5 mM KCl, 1 mM $MgSO_4$, and 2 mM $CaCl_2$

(pH 7.4) at a final volume of 1 ml. The tubes also included [³H]-spiperone in concentrations ranging from 0.05 to 0.6 nM. Non-specific binding was determined in the presence of 10 μM (---)-sulpiride. The incubation time for the assay was 30 minutes at 37° C. Incubation was terminated by vacuum filtration over glass fiber filters (Whatman GF/B, pretreated with 0.1% polyethylenimine). Filters were washed twice with ice-cold Tris-HCl buffer and radioactivity was measured by liquid scintillation spectrometry. Dopamine D₂ binding sites (B_{max}) and affinity (K_d) was determined using nonlinear regression using Prism (GraphPad Software).

Statistics

Preference scores (time spent in the non-preferred compartment on the test day minus the time spent in the same compartment on the preconditioning day), locomotor activity, D₂ binding data (i.e., B_{max} and K_d) was analyzed by separate 2 × 3 × 3 (sex × pre-exposure drug × conditioning drug) analyses of variance (ANOVA's). Tukey tests were used for post hoc comparisons. A Pearson correlation coefficient was also used to assess the relationship between the preference for the drug paired room and the number of dopamine D₂ binding sites.

CHAPTER FOUR

RESULTS

Body Weight

During the 10 days of drug administration (i.e., PD 11-20), body weight was not significantly affected by methylphenidate treatment and both males and females exhibited steady weight gain throughout the injection procedure [day main effect; $F(9, 95) = 2017.17, p < 0.001$, Tukey tests, $p < 0.05$; see Figure 1]. Body weight, however, did differ between sexes during the early drug treatment as males were significantly heavier than females on each injection day [sex \times day interaction; $F(9, 95) = 5.51, p < 0.001$; Tukey tests, $p < 0.05$].

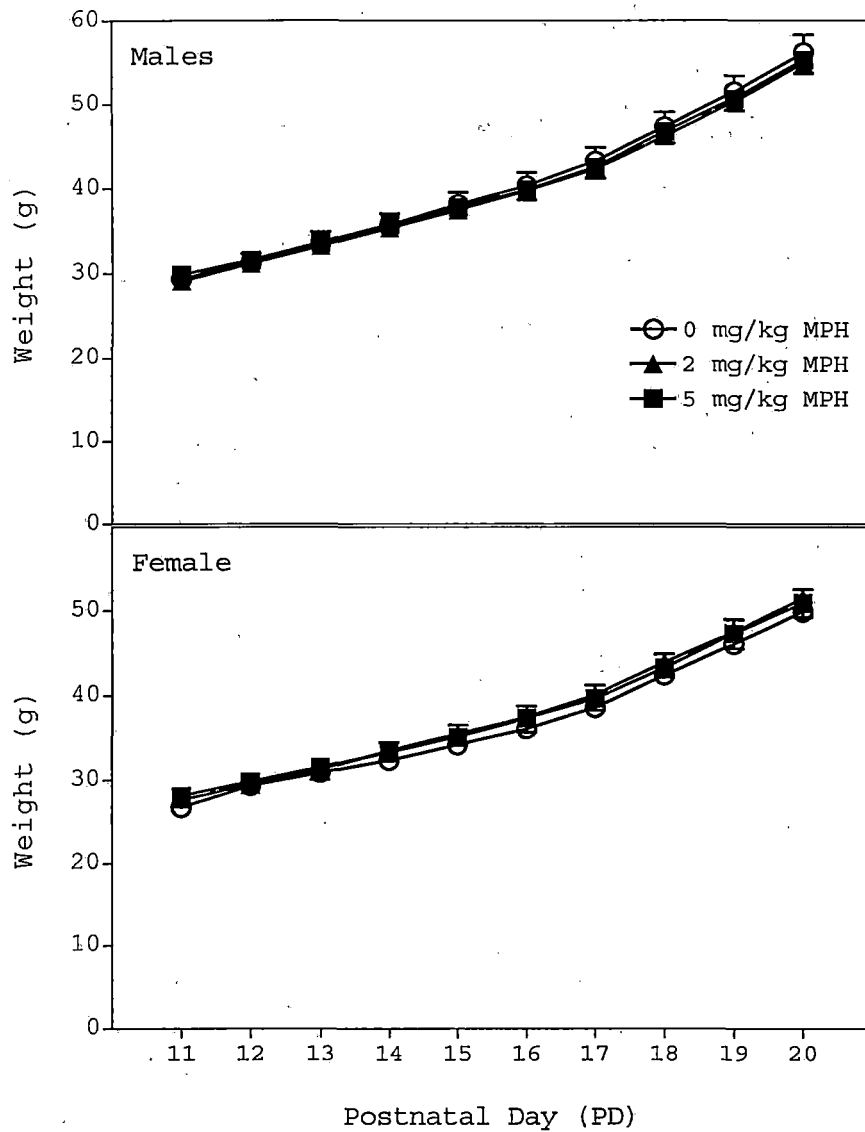


Figure 1. Mean Body Weights During the 10 Days of Methylphenidate Injections. Rats Were Injected With Methylphenidate (0, 2.0, or 5.0 mg/kg) from Postnatal Day 11 to Postnatal Day 20.

Conditioned Place Preference

Regardless of sex or pretreatment group, rats exhibited morphine-induced CPP, as rats treated with 5.0 mg/kg morphine had significantly greater difference scores than saline-treated rats [drug main effect; $F(2, 101) = 7.76, p < 0.01$; Tukey tests, $p < 0.05$; see Figure 2]. Rats treated with the lower dose of morphine (0.5 mg/kg) did not show a preference for the drug-paired room and sex did not significantly affect place conditioning. Interestingly, the preference induced by morphine was altered by methylphenidate pretreatment. Specifically, rats pretreated with 5.0 mg/kg methylphenidate had greater preference scores than rats treated with saline or 2.0 mg/kg methylphenidate [post-treatment main effect; $F(2, 32) = 4.45, p < 0.02$; Tukey tests, $p < 0.05$; see Figure 2].

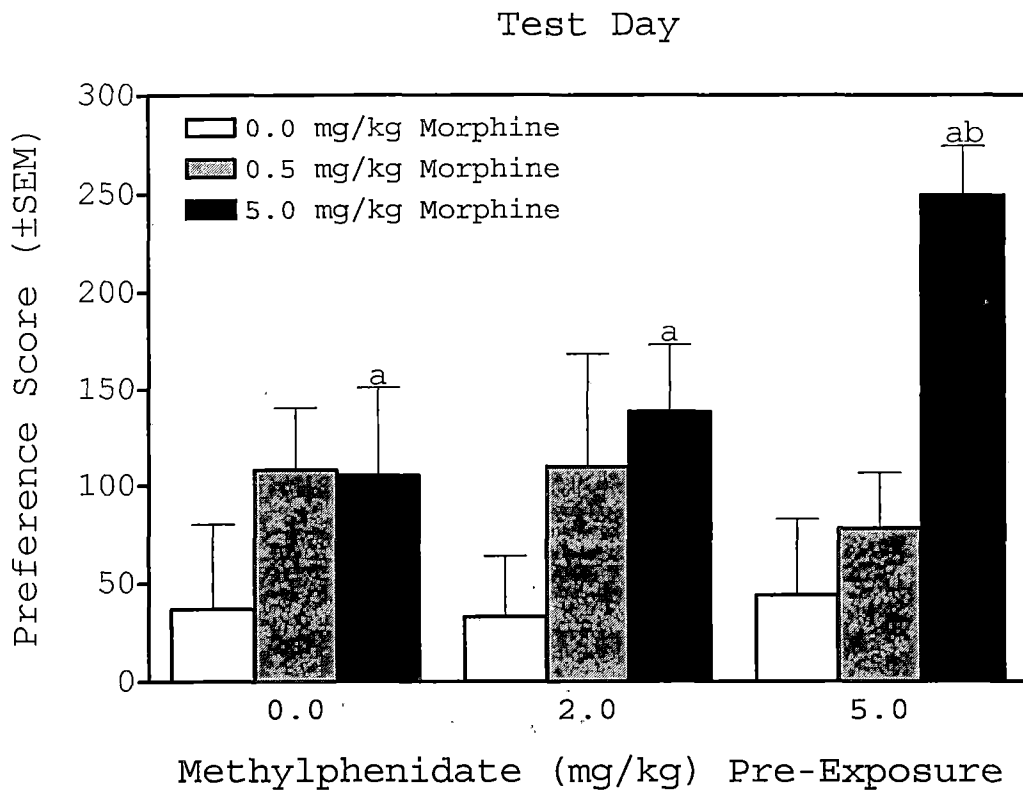


Figure 2. Mean Preference Score on Test Day. Rats Were Given Alternating Daily Injections of Morphine (0.0, 0.5, or 5.0 mg/kg) and Saline from Postnatal Day 61 to Postnatal Day 68.

^a Indicates a significant difference from rats treated with 0.0 or 0.5 mg/kg morphine (post-treatment main effect).

^b Indicates a significant difference from rats pre-exposed to 0.0 or 2.0 mg/kg methylphenidate and treated with 5.0 mg/kg morphine.

Locomotor Activity

Locomotor activity (i.e., line crosses) was assessed on the first and last day of drug administration in the drug or non-preferred chamber. Over these two testing periods, female rats expressed greater levels of locomotor activity as compared to male rats regardless of methylphenidate pretreatment or morphine exposure [sex main effect; $F(1, 89) = 5.93, p < 0.05$; see Figure 4]. Overall there were no differences in locomotor activity induced by the saline or the two doses in morphine for male or female rats. However, rats treated with 5 mg/kg morphine had similar levels of locomotor activity on both testing days whereas rats treated with saline or 0.5 mg/kg morphine had significant reductions in locomotor activity on the second day of testing [drug by day interaction; $F(2, 89) = 8.68, p < 0.01$; Tukey tests, $p < .05$; see Figure 3]. Methylphenidate pretreatment also affected locomotor activity, but only in female rats. Specifically, female rats pretreated with 2.0 mg/kg methylphenidate showed significantly less locomotor activity than similarly treated females pretreated with saline [sex by pretreatment interaction; $F(2, 89) = 4.61, p < 0.02$; Tukey tests, $p < .05$; see Figure 4].

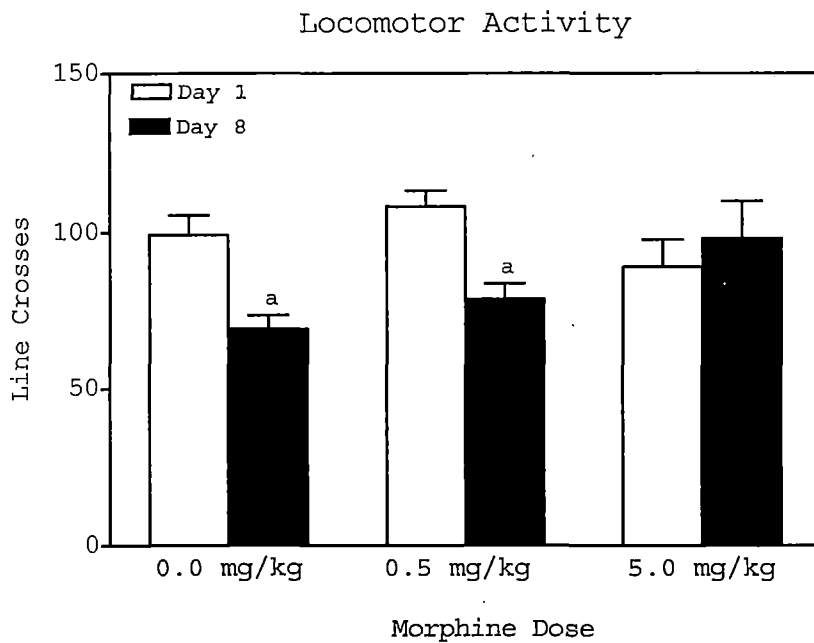


Figure 3. Mean Locomotor Activity on the First and Last Day of Drug Administration During Conditioned Place Preference Conditioning. During Conditioning, Rats Were Injected with Either 0.0, 0.5, or 5.0 mg/kg Morphine.

^a Indicates a significant difference from similarly treated rats on day 1.

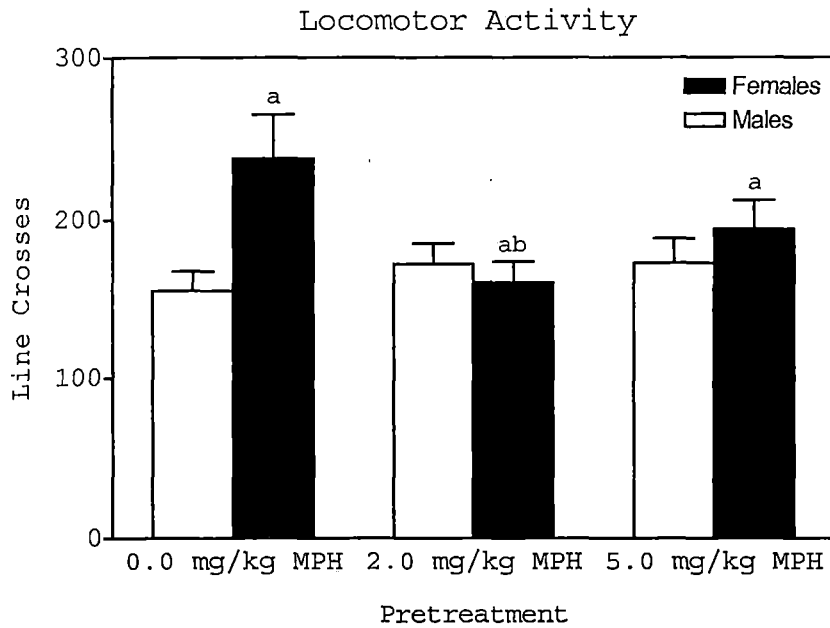


Figure 4. Mean Locomotor Activity for Male and Female Rats During Conditioned Place Preference Conditioning.

^a Indicates a significant difference between male and female rats (sex main effect).

^b Indicates a significant difference from female rats pretreated with 0.0 mg/kg methylphenidate.

D₂ Receptor Binding

Neither D₂ binding densities (i.e., B_{max}) or affinity (i.e., K_D) were altered by methylphenidate pretreatment or morphine exposure. However, there was a significant positive correlation between the number of D₂ binding sites and morphine-induced preference scores ($r = 0.32, p < 0.05$, see Figure 5). When this relationship was examined within each pretreatment group, a positive correlation was only found for rats in the saline and 5.0 mg/kg methylphenidate pretreatment groups ($r = .62, p < .05$; $r = .82, p < .01$, respectively; see Figures 6 and 7), but not for rats pretreated with 2.0 mg/kg methylphenidate. Because rats conditioned with saline and 0.5 mg/kg of morphine failed to exhibit a conditioned place preference, only rats in the 5.0 mg/kg condition were used in the analysis.

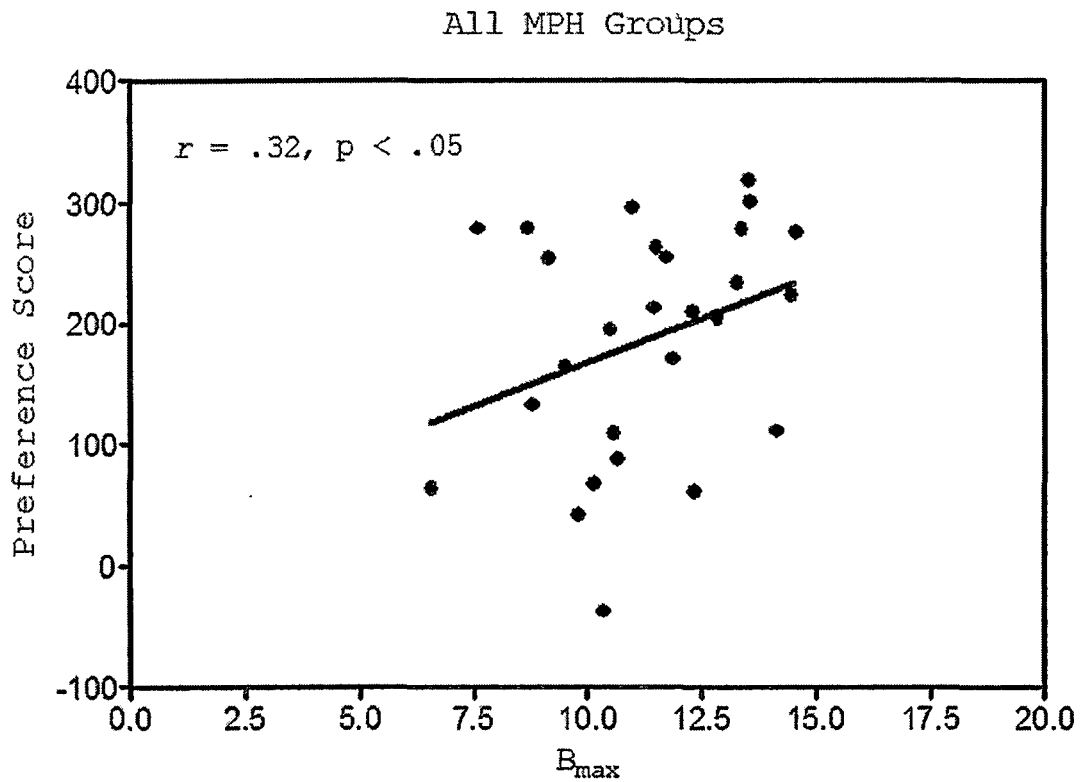


Figure 5. Correlation Between Preference Scores and Number of Dopamine D₂-Like Binding Sites in Rats Pretreated with Methylphenidate (0.0, 2.0, or 5.0 mg/kg) from Postnatal Day 11 to Postnatal Day 20 and Conditioned with 5 mg/kg Morphine.

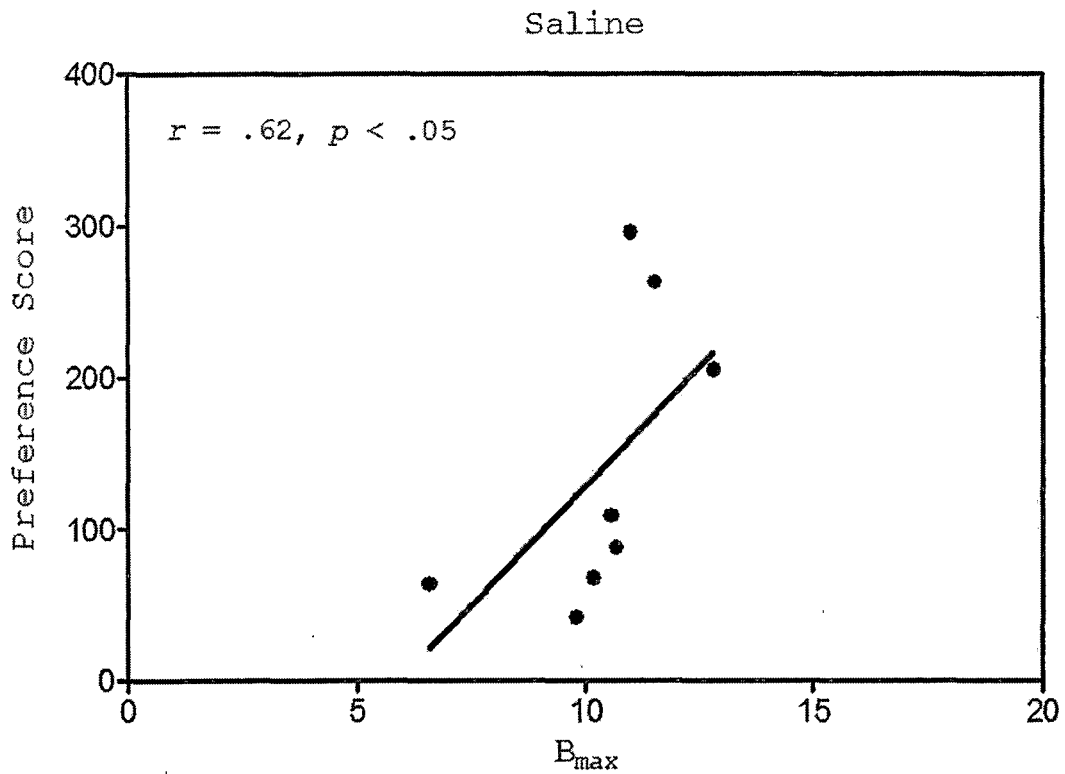


Figure 6. Correlation Between Preference Scores and Number of Dopamine D₂-Like Binding Sites in Rats Pretreated with Saline from Postnatal Day 11 to Postnatal Day 20 and Conditioned with 5 mg/kg Morphine.

5 mg/kg MPH

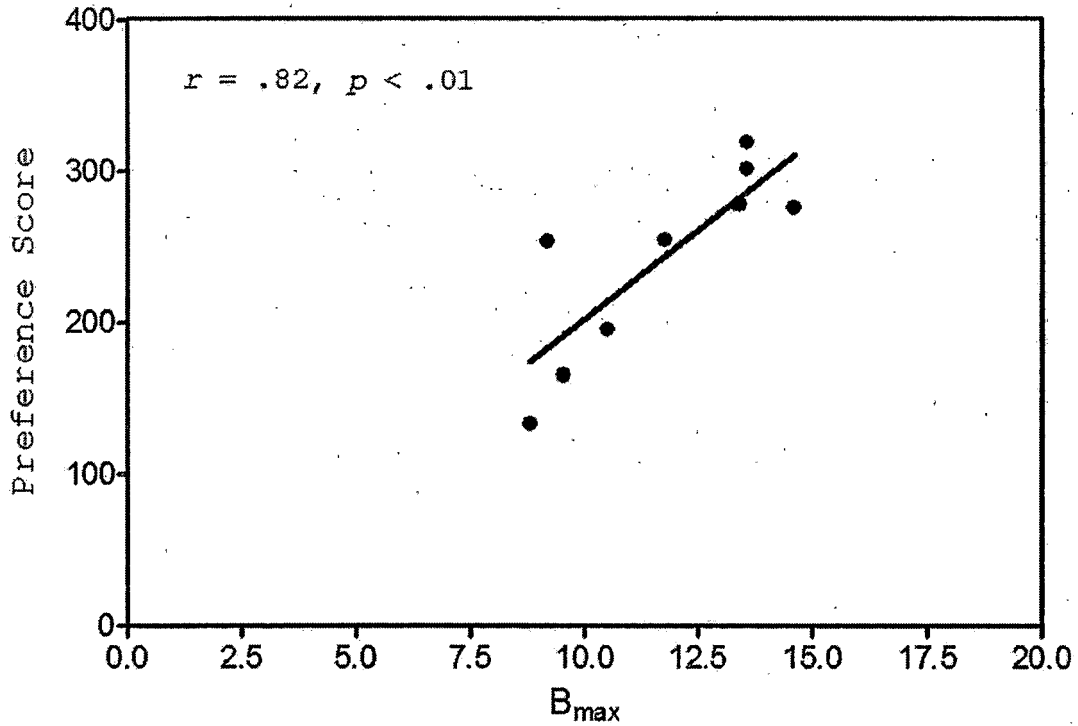


Figure 7. Correlation Between Preference Scores and Number of Dopamine D₂-Like Binding Sites in Rats Pretreated with 5.0 mg/kg Methylphenidate from Postnatal Day 11 to Postnatal Day 20 and Conditioned with 5 mg/kg Morphine.

CHAPTER FIVE

DISCUSSION

Overview

The purpose of the present study was to determine whether chronic treatment with methylphenidate during the preweanling period would alter the reinforcing properties of morphine in young adult rats. To this end, we determined whether early exposure to methylphenidate would alter morphine-induced CPP in young adult rats. In addition, we also examined whether dopamine D₂ binding sites would be affected by early methylphenidate treatment and if the number of sites would be correlated with morphine-induced CPP. Two hypotheses were made regarding the effects of early methylphenidate treatment on morphine-induced CPP and D₂ binding sites. Specifically, it was predicted that rats pre-exposed to methylphenidate would: (1) have greater preference scores as compared to saline pre-exposed rats, and (2) have fewer D₂ receptors than saline exposed rats. In addition, we hypothesized that there would be a negative correlation between preference scores and the number of D₂ binding sites.

Effects of Methylphenidate Pre-Exposure
on Morphine-Induced Conditioned Place
Preference

Consistent with numerous other reports, young adult rats in the present study exhibited a morphine-induced CPP, as rats spent significantly more time in the initially non-preferred compartment after morphine conditioning as compared to saline treated rats (for reviews see Bardo et al., 1995; McBride et al., 1999). However, this increased preference for the drug-paired compartment was only observed in rats conditioned with the high dose of morphine (i.e., 5 mg/kg), but not the low dose (i.e., 0.5 mg/kg). This dose effect was not unexpected as a previous meta-analysis of morphine-induced CPP showed that doses between 1 and 10 mg/kg were fairly equivalent in producing place preference, while doses under 1 mg/kg were found to be suboptimal (Bardo et al., 1995).

Early exposure to 5 mg/kg methylphenidate, as originally predicted, increased the magnitude of the morphine-induced CPP. This result suggests that exposure to methylphenidate during the preweanling period increases the rewarding value of morphine. This conclusion is consistent with studies showing that exposing adolescent rats and mice to methylphenidate enhances both cocaine self-administration and cocaine-induced CPP (Achat-Mendes et al., 2003; Brandon

et al., 2001). However, these results contrast with studies using preadolescent rats, in which early methylphenidate exposure decreased cocaine-induced CPP (Andersen et al., 2002; Carlezon et al., 2003). Taken together, it appears that age at drug exposure may be critical for determining the long-term impact of methylphenidate. Specifically, methylphenidate exposure during the preadolescent period may decrease later drug responsiveness, while exposing rats to methylphenidate during earlier (preweanling) or later (adolescent) developmental periods may enhance drug responsiveness.

Interestingly, enhanced morphine-induced CPP was only observed in rats pre-exposed to the 5 mg/kg dose of methylphenidate. This dose effect was not anticipated, as a previous study using adolescent rats had found that 2 mg/kg methylphenidate was sufficient to enhance the rewarding effects of cocaine in a self-administration paradigm. This may be suggestive of an age-dependent difference in the sensitivity to repeated methylphenidate treatment (i.e., adolescent rats are more sensitive than preweanling rats). Alternatively, the self-administration paradigm may be a more sensitive tool for assessing changes in reward value.

Early Methylphenidate Pre-Exposure and D₂ Receptors

The present study, as well as the previously mentioned developmental methylphenidate studies, demonstrated that early methylphenidate exposure can alter drug-rewarded behavior. The neurobiological cause of this alteration in rewarded behavior is unknown, but changes involving D₂ receptors may be responsible. Evidence for D₂ receptor involvement is threefold: first, several studies using D₂ antagonists and D₂ knock-out mice have demonstrated the importance of D₂ receptors for drug-rewarded behaviors (Di Chiara, 1999; Risinger, Freeman, Rubinsten, Low, & Grandy, 2000; Salamone, Cousins, & Snyder, 1997; Noble, 1996; Smith, Fetsko, Xu, & Wang, 2002); second, early exposure to methamphetamine (a psychostimulant similar to methylphenidate) has been reported to cause a decline in D₂ receptors that is detectable in adulthood (Crawford et al., 2003); and third, human imaging studies have demonstrated that addiction to psychostimulants and other abused drugs is associated with lower levels of D₂ receptors and that the number of striatal D₂ receptors in nondrug-abusing subjects is negatively correlated with the reinforcing value of methylphenidate (Volkow et al., 2002; 2004). Thus, based on evidence from both the animal and human literature, we

predicted that D₂ receptor binding sites would decline after early methylphenidate treatment, thus providing a mechanism for changes in morphine-induced reward. Moreover, we hypothesized that the number of D₂ receptors would be negatively correlated with morphine-induced preference scores.

Our hypothesis regarding changes in D₂ receptor sites was not supported, because we found that the number of D₂ receptors was not effected by early methylphenidate exposure. Interestingly, however, the number of D₂ receptors was positively correlated with morphine-induced preference scores. It is not known why morphine-induced CPP was positively correlated with D₂ receptors, while the rewarding effects of other drugs (i.e., methylphenidate and alcohol) were found to have negative relationship to D₂ receptors is unknown (Volkow et al., 2002; 2004). It is possible that exposure to morphine changed the number of D₂ binding sites. Alternatively, the positive association between D₂ binding sites and morphine-induced CPP may be applicable to only morphine and not other drugs of abuse. This alternative is supported by a study showing that D₂ knock-out mice do not acquire morphine-induced CPP, but they do show normal cocaine-induced CPP (Smith et al., 2002),

suggesting that morphine-induced CPP is dependent on D₂ receptors while cocaine-induced CPP is not.

Implications and Conclusions

In accordance with previous research, the present study provides additional evidence that early exposure to methylphenidate causes behavioral adaptations that persist into adulthood. Our results show that exposure to methylphenidate during the preweanling period increases the rewarding value of morphine. These results suggest that treating young children (e.g. preschool age) with methylphenidate may be putting them at risk for drug addiction later in life.

The finding that higher levels of D₂ receptors were associated with morphine-induced CPP may reflect inadequacies in the current dopamine deficiency theory of addiction. The current understanding is that decreased CPP should be associated with higher levels of D₂ receptors and increased CPP is associated with lower levels of D₂ receptors. This association does not hold true for our findings. It may be possible that the theory does not apply to morphine-induced reward, because greater preference scores should also be exhibited by rats treated with saline that have higher levels of D₂ receptors.

In summary, rats pretreated with methylphenidate showed greater preference scores than saline pretreated rats. In particular, early exposure to 5 mg/kg methylphenidate increased the magnitude of morphine-induced CPP, which supports our original hypothesis and suggests that exposure to methylphenidate in the preweanling period increases the rewarding value of morphine. This finding indicates an age-dependent mechanism for assessing long-term drug exposure. This evidence presupposes that preweanling rats exposed to methylphenidate can alter drug-rewarded behavior and enhance drug responsiveness in adulthood and suggests that treating young children (e.g. preschool age) with methylphenidate may increase the risk of drug addiction later in life. Our second hypothesis was not supported; early exposure to methylphenidate did not change the number of D₂ receptors. We had originally predicted that an increase in morphine-induced CPP would also be associated with decreased levels of D₂ receptors, our negative finding has implications for the current understanding of morphine-induced reward in regards to the dopaminergic theory of addiction. In conclusion, the well known link between ADHD and subsequent substance abuse (Wilens, Biederman, Mick, Faraone, & Spencer, 1997; Clure, Brady, Saladin, Johnson, Waid, & Rittenbury, 1999; Schubiner et al., 2000), coupled with the

relative dearth of controlled studies on the long-term effects of methylphenidate in young subjects, is emerging as an important public health issue which requires further study.

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