Effect of preweanling methylphenidate exposure on the induction, extinction and reinstatement of morphine-Induced conditioned place preference in rats

Kellie Lynn Kucher

Follow this and additional works at: https://scholarworks.lib.csusb.edu/etd-project

Part of the Biological Psychology Commons

Recommended Citation
https://scholarworks.lib.csusb.edu/etd-project/2892

This Thesis is brought to you for free and open access by the John M. Pfau Library at CSUSB ScholarWorks. It has been accepted for inclusion in Theses Digitization Project by an authorized administrator of CSUSB ScholarWorks. For more information, please contact scholarworks@csusb.edu.
EFFECT OF PREWEANLING METHYLPHENIDATE EXPOSURE ON THE INDUCTION, EXTINCTION AND REINSTATEMENT OF MORPHINE-INDUCED CONDITIONED PLACE PREFERENCE IN RATS

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
Kellie Lynn Kucher
June 2005
EFFECT OF PREWEANLING METHYLPHENIDATE EXPOSURE ON
THE INDUCTION, EXTINCTION AND REINSTATEMENT OF
MORPHINE-INDUCED CONDITIONED PLACE PREFERENCE
IN RATS

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

by
Kellie Lynn Kucher
June 2005

Approved by:

Cynthia Crawford, Chair, Psychology

Sanders McDougall

Frederick A. Newton

3/09/05
ABSTRACT

The clinical use of methylphenidate for pre-school aged children has become increasingly common over the last decade. However, little is known about the long-term effects of stimulant medication on this age group. Developmental studies in rodents suggest that early methylphenidate exposure may alter later reward to drugs of abuse and cause alterations in neuronal functioning. These studies have generated conflicting results, with some studies suggesting that methylphenidate decreases later drug reward while other studies indicating methylphenidate increases later drug reward. The present study examined the effect of preweanling methylphenidate exposure on later drug reward. To this end, we examined the induction, extinction, and reinstatement of morphine-induced conditioned place preference (CPP) in rats that received methylphenidate pretreatment during the preweanling period. It was predicted that rats pretreated with methylphenidate would show a greater preference for morphine. In addition, methylphenidate pretreated rats were predicted to extinguish more slowly and show a greater morphine-induced CPP after reinstatement. The results of our study indicate that preweanling methylphenidate exposure does affect
morphine CPP. While methylphenidate pretreated rats did not show an initial preference for morphine, the other two predictions were supported. Rats pretreated with the high dose of methylphenidate were slower to extinguish as compared to rats pretreated with the low dose of methylphenidate or saline. In addition, CPP was more easily reinstated in rats pretreated with the high dose of methylphenidate than rats pretreated with the low dose of methylphenidate or saline. These findings have implications for the use of methylphenidate in pre-school aged children as a risk factor for vulnerability towards drug abuse. Our findings indicate that early methylphenidate exposure increases later drug reward, therefore increasing vulnerability towards drug abuse. One may be more susceptible towards drug addiction because drugs of abuse become more rewarding following stimulant treatment in early childhood.
ACKNOWLEDGEMENTS

I would like to thank all those involved in contributing to the completion of my thesis. First and foremost, I would like to thank my thesis advisor, Dr. Cynthia Crawford, for her expertise, feedback and encouragement. In addition, I want to thank the additional members of my thesis committee, Dr. Sanders McDougall and Dr. Frederick A. Newton. Your insight and guidance are greatly appreciated. It has truly been a privilege to work with all members of my thesis committee. Additionally, I’d like to express my appreciation to my graduate coordinator, Dr. McDougall, for his efforts in helping me achieve my master’s degree. Lastly, I am grateful to my research assistants, Chad Andicochea, Jeffrey Proctor, and Cristal Farley for their hard work and commitment to my thesis project.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
<tr>
<td>CHAPTER ONE: STIMULANT MEDICATION IN EARLY DEVELOPMENT</td>
<td></td>
</tr>
<tr>
<td>Attention-Deficit/Hyperactivity Disorder</td>
<td>1</td>
</tr>
<tr>
<td>Stimulant Medication to Treat</td>
<td>1</td>
</tr>
<tr>
<td>Attention-Deficit/Hyperactivity Disorder</td>
<td></td>
</tr>
<tr>
<td>Stimulant Medication Effectiveness</td>
<td>4</td>
</tr>
<tr>
<td>Preschool-Aged Children and Attention-Deficit/Hyperactivity Disorder</td>
<td>5</td>
</tr>
<tr>
<td>Preschool-Aged Children and Stimulant Medication</td>
<td>8</td>
</tr>
<tr>
<td>Stimulant Side Effects in Attention-Deficit/Hyperactivity Disorder</td>
<td>11</td>
</tr>
<tr>
<td>Stimulant Treatment as a Risk for Substance Abuse Disorder</td>
<td>14</td>
</tr>
<tr>
<td>CHAPTER TWO: NEUROTOXICITY OF AMPHETAMINIES</td>
<td></td>
</tr>
<tr>
<td>Neurotoxic Potential of Methylphenidate</td>
<td>20</td>
</tr>
<tr>
<td>Evidence for Amphetamine Neurotoxicity</td>
<td>21</td>
</tr>
<tr>
<td>Free Radicals Theory of Amphetamine Neurotoxicity</td>
<td>24</td>
</tr>
<tr>
<td>Dopamine Is Essential for Neurotoxicity</td>
<td>27</td>
</tr>
<tr>
<td>Conclusion</td>
<td>28</td>
</tr>
</tbody>
</table>
CHAPTER THREE: DEVELOPMENTAL DIFFERENCES IN AMPHETAMINE NEUROTOXICITY

Resistance to Methamphetamine Neurotoxicity in Young Rats .................................. 29

Hyperthermia in Methamphetamine Neurotoxicity ......................................................... 31

Hyperthermia in Resistance to Methamphetamine Neurotoxicity in Young Rats ............. 32

CHAPTER FOUR: ANIMAL MODELS OF DRUG REWARD

Introduction ................................................. 35

Reward Versus Reinforcement ......................... 36

Self-Administration Paradigm ......................... 38

Conditioned Place Preference Paradigm ............. 39

What Is Learned in Conditioned Place Preference? ......................................................... 41

Comparison Between Self-Administration and Conditioned Place Preference .......... 43

Are Conditioned Place Preference and Self-Administration Comparable Methods for Measuring Reward? ......................................................... 50

CHAPTER FIVE: THE DOPAMINE SYSTEM AND REWARD

Introduction ................................................. 54

Self-Administration Studies ......................... 54

Conditioned Place Preference Studies ............. 57

Brain Self-Stimulation Studies ....................... 58

Other Drugs of Abuse Stimulate Dopamine Release ....................................................... 60

Conclusion .................................................. 61
CHAPTER SIX: AMPHETAMINE IMPACTS REWARD

Introduction ........................................... 62

Behavioral Research: Evidence that Amphetamine Impacts Reward .................. 63

The Dopamine D₂ Receptor Hypothesis: The Role of the Dopamine D₂ Receptor in the Ability of Amphetamine to Impact Reward .............. 66

Evidence that Amphetamine Alters Dopamine D₂ Receptors ............................... 66

Dopamine D₂ Receptor Levels Predict Reinforcing Response to Drugs ................. 69

Conclusion .................................................. 71

CHAPTER SEVEN: EARLY AMPHETAMINE EXPOSURE AFFECTS REWARD AND LATER BEHAVIOR

Early Amphetamine Exposure Affects Reward .................................................. 73

Early Amphetamine Exposure Affects Later Behavior ......................................... 76

CHAPTER EIGHT: SUMMARY AND HYPOTHESIS ............................................. 78

CHAPTER NINE: METHODS

Subjects ..................................................... 83

Drugs ......................................................... 83

In Vivo Drug Treatment ........................................ 83

Conditioned Place Preference Apparatus ....................................................... 84

Conditioned Place Preference Procedure ....................................................... 85

Preconditioning Day ........................................ 85

Conditioning Phase ........................................ 85

viii
Test for Induction of Morphine-Induced Conditioned Place Preference .......... 86
Extinction Phase .......... 86
Test for Extinction of Morphine-Induced Conditioned Place Preference .......... 87
Test for Reinstatement of Morphine-Induced Conditioned Place Preference .......... 87
Experimental Design and Statistical Analysis ........................................ 88
Body Weight .......... 88
Preconditioning Compartment Preference .......... 88
Induction of Conditioned Place Preference .......... 89
Extinction of Conditioned Place Preference .......... 89
Reinstatement of Conditioned Place Preference .......... 89
Locomotor Activity on the First and Last Day of Morphine Exposure .......... 90

CHAPTER TEN: RESULTS

Preweanling Body Weights .......... 91
Adult Body Weights .......... 91
Initial Chamber Preference .......... 93
Induction of Conditioned Place Preference .......... 94
Extinction of Conditioned Place Preference .......... 96
CHAPTER ELEVEN: DISCUSSION

Effects of Preweanling Methylphenidate
Preweanling Exposure on Morphine-Induced
Reward in Early Adulthood .......................... 102

Implications for Use of Methylphenidate
for the Treatment of Attention-Deficit/
Hyperactivity Disorder in Early Childhood
with Regard to Vulnerability Toward
Drug Abuse ............................................. 109

REFERENCES ............................................ 113
LIST OF FIGURES

Figure 1. Mean Preweanling Body Weights During 10 Days of Methylphenidate Injections ............................ 92

Figure 2. Mean Body Weights (± SEM) on the First Day of Conditioned Place Preference Training (PND 61) for Male and Female Rats Previously Exposed to Methylphenidate (0, 2, 5 mg/kg) from PND 10 to PND 19 ........................ 93

Figure 3. Mean Time Spent in Each Compartment (± SEM) on the Initial Preference Day (PND 60) ............................ 94

Figure 4. Mean Time Spent in Each Compartment (± SEM) on the Induction Test Day (PND 68) ............................ 95

Figure 5. Mean Time (± SEM) Spent in the Drug-paired Compartment on the Extinction Test Day (PND 77) ............................ 98

Figure 6. Mean Time (± SEM) Spent in the Drug-paired Compartment on the Reinstatement Test Day (PND 78) ............................ 99

Figure 7. Mean Line Crosses (± SEM) on the First and Last Days of Conditioned Place Preference Conditioning ............................ 101
CHAPTER ONE

STIMULANT MEDICATION IN

EARLY DEVELOPMENT

Attention-Deficit/
Hyperactivity Disorder

Attention-deficit hyperactivity disorder (ADHD) is the most common neuropsychiatric diagnosis given to children, with currently 3% - 5% of children meeting the DSM-IV diagnostic criteria for the disorder (Kwasman, Tinsley & Lepper, 1995). The disorder disrupts central nervous system regulation of attention span, impulsiveness, and motor activity, and presents significant challenges to affected children, their families, and the school system (Kwasman et al., 1995). Given the high prevalence of this problem, along with its major impact on quality of life, it is imperative that we study its possible etiologies, clinical manifestations, and treatments (Palfrey, Levine, Walker & Sullivan, 1985).

Stimulant Medication to
Treat Attention-Deficit/
Hyperactivity Disorder

Outpatient visits devoted to ADHD increased from 1.6 to 4.2 million per year during the years of 1990-1993.
(Swanson, Lerner & Williams, 1995). During those visits, 90% of the children were given prescriptions, 71% of which were for the stimulant, methylphenidate. Methylphenidate production in the United States increased from 1,784 kg to 5,110 kg during the same time period, so that over 10 million prescriptions for methylphenidate were written in 1996 (Vitiello & Jensen, 1997).

Stimulant medication remains the most common way to treat ADHD. One study surveyed randomly selected members of the American Academy of Pediatrics (AAP) concerning ADHD assessment and treatment (Copeland, Wolraich, Lindgren, Millch & Woolson, 1987). The respondents in the survey reported that their most frequently used therapy for ADHD was methylphenidate. In a follow up to this study, children, physicians, parents and teachers were intensively interviewed in an effort to ascertain the treatment used for children with ADHD (Wolraich et al., 1990). Findings from this study indicated that stimulant medication, particularly methylphenidate, was the treatment of choice for ADHD by family practice physicians. More recently, in a national survey of 380 members of the American Academy of Pediatrics, respondents reported that 50% of their patients had not received educational testing before they wrote...
prescriptions for methylphenidate and similar drugs, and even fewer of their patients received psychological testing (Kwasman et al., 1995). In terms of medications that the pediatricians reported using in treating ADHD, methylphenidate was reported as being prescribed 97.6% of the time (Kwasman et al., 1995). Finally, in the only study that used a nationally representative sample of patient records from office-based physicians, it was found that over 26% of the children receiving psychotropic medications were not scheduled for follow-up visits and only 36% were provided any counseling or psychotherapy (Kelleher, Hohmann & Larson, 1989). The 1996 Practice Parameters for the Assessment and Treatment of Children, Adolescents, and Adults with ADHD cite three "cornerstones" of treatment: parent support and education, appropriate school placement, and pharmacology. However, as evidenced in the research, it appears that stimulant medication is the predominant and preferred method of treatment for ADHD, to the extent that other treatment modalities have been neglected. Because stimulant medication is prescribed so readily for ADHD, it is important that the possible aversive long-term consequences be examined.
Stimulant Medication
Effectiveness

Although there are few long-term studies, stimulant medication has been proven effective in reducing ADHD symptoms. Stimulants improve disruptive ADHD behaviors in the home, classroom, and playground (Solanto, Arnsten & Castellanos, 2001). At home, stimulants improve compliance and parent-child interactions (Whalen et al., 1989). In the classroom, stimulants increase on-task behavior and decrease interrupting and restlessness (Abikoff & Gittelman, 1985). On the playground, stimulants reduce covert aggression (Hinshaw, Heller & McHale, 1992), overt aggression (Gadow, Nolan, Sverd, Sprafkin & Paolicelli, 1990), and symptoms of conduct disorder (Klein et al., 1997) and increase attention during sports (Pelham et al., 1990). Stimulants decrease impulsive responding and response variability on cognitive tasks (Tannock, Schachar & Logan, 1995); increase accuracy of performance, improve sustained attention, short-term memory, and reaction time (Hinshaw, Henker, Whalen, Ehrardy & Dunnington, 1989). In addition to treating ADHD symptoms, stimulant medications are beneficial in treating other medical conditions, such as narcolepsy and depression (Goldman, Genei, Bazman &
Stanetz, 1998). Because stimulant medication is so successful in reducing ADHD symptoms, it is understandable why physicians use it as the predominant way to treat ADHD. The general consensus in the medical community is that the effectiveness of stimulant medication in treating ADHD outweighs its side effects. However, because the possible long-term aversive effects have not been studied thoroughly, it is important that research continue to examine this possibility. One major concern is whether stimulant treatment produces neurotoxic effects that would later increase vulnerability towards drugs of abuse.

Preschool-Aged Children and Attention-Deficit/Hyperactivity Disorder

The signs and symptoms of ADHD are now believed to be evident before the age of 3 (Solanto et al., 2001). Even though ADHD-related behaviors displayed by preschool age children resemble behaviors among older ADHD patients, the diagnostic manuals give little guidance about the validity of ADHD diagnosis in the preschool years. School-age norms gathered on standard teacher global rating forms, such as the Conners Teacher Questionnaire (CTQ), have not included preschoolers until recently (Solanto et al., 2001). For
many reasons, the clinical diagnosis of ADHD in preschool children is challenging. The first problem in diagnosis is the nonspecificity of ADHD symptoms in the 2- to 5-year old range. The core symptoms of ADHD – inattention, impulsivity, and overactivity are common daily behaviors of most preschool aged children. Studies have shown that up to 40% of children by the age of 4 years have enough problems with inattention to cause concern to their parents and preschool teachers (Palfrey et al., 1985). Yet studies also show that the vast majority of these concerns are short-lived and generally diminish within 3 to 6 months. Even among those children whose symptoms are severe and frequent enough to justify a diagnosis of ADHD in the preschool years, only 48% will have the same diagnosis by later childhood or adolescence. These findings suggest that the appearance of significantly inattentive or overactive behaviors by age 3 to 4 years, by themselves, is not indicative of a persistent pattern of ADHD in later childhood or adolescence in at least 50% of preschool children (Barkley, 1998). However, there are those preschool age children who do display ADHD symptoms and do warrant the diagnosis and treatment for ADHD. Approximately 5% to 10% of preschoolers with parental or
teacher concerns about inattention eventually develop a pattern of persistent inattention consistent with ADHD by the second grade (Palfrey et al., 1985). One study characterized a sample of preschool aged children as either 'true' hyperactive or 'situationally' hyperactive (Campbell, Endman & Bernfield, 1977). 'True' hyperactive children were characterized by cross-situational high activity while 'situationally' hyperactive children were those who were situation-specific hyperactive, with their hyperactivity being observed only in the home. They found that children classified as 'true' hyperactive in the preschool years continued to manifest problems in elementary school as measured by classroom observation and teacher ratings. In assessing preschoolers who display significant attentional or behavioral difficulties, the clinical task is to distinguish between the 5% to 10% who will develop ADHD and the 90% to 95% who have developmentally appropriate and temporary symptoms of ADHD-like symptoms from other causes. Thus, the degree of ADHD symptoms, their pervasiveness across settings, and their duration determine which children with early-onset difficulties are likely to show a chronic course of their ADHD symptoms throughout development (Barkley, 1998).
Preschool-Aged Children and Stimulant Medication

Although research has indicated that diagnosing ADHD in the early years is difficult, the clinical use of stimulant medication for 3- to 6- year old preschool children who meet the diagnostic criteria for ADHD is becoming more common. There is a growing concern about the increasing numbers of young children being treated with stimulants (Safer & Zito, 1996). Unfortunately, there is a lack of research assessing stimulant effects on the very young and developing brain.

Since 1975, only nine double-blind placebo-controlled studies have assessed the efficacy of stimulants for ADHD in preschool children 1.8 to 6 years of age (Connor, 2002). All nine studies assessed ADHD children on methylphenidate. No other type of stimulant (Adderall, Concerta, dextroamphetamine, or pemoline) has been assessed under controlled conditions in the preschool age range. Studies have assessed the efficacy of methylphenidate in alleviating ADHD symptoms by using reports from parents, caregivers, and nursery school personnel. Only one controlled study has assessed outcome using laboratory psychological tests in this age group (Byrne, Bawden,
DeWolfe & Beattie, 1998). The two neuropsychological domains assessed in methylphenidate drug studies of preschool ADHD children are cognition and attention span. Behavioral domains assessed include hyperactivity/impulsivity and interpersonal interactions. In eight of the nine controlled studies, 89% report methylphenidate as effective in treating the symptoms of ADHD in preschool children (Barkley, Karlsson, Strzelecki & Murphy, 1984; Barkley, 1988; Byrne et al., 1998; Conners, 1975; Cunningham, Siegel & Offord, 1985; Handen, Feldman & Lurier, 1999; Mayes, Crites, Bixler, Humphrey & Mattison, 1994; Monteiro-Musten, Firestone & Pisterman, 1997). Three studies report improvements across all domains including behavioral, interpersonal, and cognitive functioning (Byrne et al., 1998; Cunningham et al., 1985; Monteiro-Musten et al., 1997). Improvement is reported in two studies specifically assessing behavioral domains (Conners, 1975; Mayes et al., 1994) and in two studies investigating interpersonal interactions in ADHD preschoolers (Barkley et al., 1984; Barkley, 1988).

However, not all methylphenidate studies report benefits of ADHD children in the 3- to 6-year old range. One controlled study found mixed results with many reported
side effects using methylphenidate in preschool ADHD children (Schleifer et al., 1975). One study randomly assigned children to either methylphenidate, cognitive behavioral therapy, or no treatment. Results did not demonstrate treatment benefits for either methylphenidate or behavioral therapy relative to no treatment for 24 ADHD preschool children (Cohen, Sullivan, Minde, Novak & Helwig 1981). Another controlled study, although reporting positive effects of drugs on ADHD symptoms in preschoolers, noted a large variability in individual responses (Conners, 1975). Although not all studies agree, these controlled studies indicate that methylphenidate in the preschool age range is beneficial in the treatment of ADHD.

However, there are considerable limitations in the research literature. The first limitation is the small number of methodologically controlled studies (nine) and the small sample sizes of studies assessing stimulant efficacy for ADHD in the preschool years. Of the 5768 children, adolescents, and adults studied under controlled conditions in stimulant drug trials for ADHD, only 206 subjects are in the preschool-age range (Spencer et al., 1996). In order to draw conclusions about the safety and
efficacy of stimulants for preschool ADHD children, much more controlled research is necessary.

Also, the duration of methylphenidate drug trials in the preschool years has been brief. The average length of methylphenidate therapy in these studies is only a little longer than 4 weeks. Because ADHD is commonly a chronic neuropsychiatric disorder that may last several years, and that preschool ADHD children may be treated with stimulants for many years, there is a need for studies assessing long-term stimulant treatment safety and efficacy when stimulant medication for ADHD is given early in development (Connor, 2002).

Stimulant Side Effects in Attention-Deficit/ Hyperactivity Disorder Preschoolers

As the diagnosis of ADHD and use of stimulant medication become more common in the preschool years, there is concern about the possible side effects of treating very young children with these medications. Because studies done in the 1970's showed many side effects in ADHD preschoolers treated with methylphenidate, the general clinical opinion has been that very young ADHD children experience more frequent and possibly more severe side
effects of stimulant medication than older ADHD elementary-
school children (Conners, 1975; Schleifer et al., 1975).

Recently, researchers have begun to systematically
evaluate side effects of stimulant drug treatment in ADHD
preschoolers in methodologically controlled designs. There
are two studies presently available (Barkley et al., 1990;
Firestone, Monteiro-Musten, Pisterman, Mercer & Bennett,
1998). These two studies used the same side-effects rating
scales, methylphenidate dosing, and methodological design.
One study assessed methylphenidate side effects on a 17-
item rating scale in school-aged children 5- to 13-years
old (Barkley et al., 1990). The other study investigated
methylphenidate on the same 17-item rating scale in
preschool children 4-to 6-years of age (Firestone et al.,
1998). Doses of methylphenidate used in both studies were
placebo, 0.3 mg/kg and 0.5 mg/kg given twice daily. Both
studies used a blinded, placebo-controlled crossover design
in which children were randomized to each drug condition
for 7 to 10 days before crossing over into the next drug
condition.

By comparing these two studies, several points about
methylphenidate side effects can be noted. First, ADHD
children receiving placebo were found to have many of the
behavioral side effects attributable to methylphenidate. Second, methylphenidate side effects in preschool and school-aged children are generally described as mild. Third, this comparison of the two studies suggests the possibility that methylphenidate side effects reported as severe by parents may be slightly increased in preschool ADHD children (10%) as compared to older children (3.6%). Fourth, behaviors reported as side effects may actually improve on drug treatment (i.e. insomnia, anxiety, and irritability in ADHD preschoolers). Lastly, except for appetite suppression, side effects reported as significant in younger ADHD children (sad, nightmares, drowsy, talks less, uninterested) are not the same side effects reported as significant for older school-aged ADHD children (insomnia, stomachache, headache). This suggests that type, frequency, and/or severity of methylphenidate-induced side effects may change with age and development.

Before conclusions can be made, much more research is needed that compares safety and efficacy of stimulants across development. Unanswered questions involve the long-term safety of stimulant medication for preschool-aged children and the longer duration of treatment over the developing years. It is important to consider the
possibility of adverse effects of stimulant medication on the developing brain. The CNS structures believed to be important in the regulation of attention span and motor activity include the prefrontal cortex, thalamus, basal ganglia, and cerebellum (Solanto, 1998). Many of these structures mature well after birth. Thus, stimulant treatment during early development may alter the development of these important brain structures. One area of particular interest is whether early methylphenidate treatment would alter the brain in such a way as to increase later vulnerability to drugs of abuse.

Stimulant Treatment as a Risk for Substance Abuse Disorder

It is difficult to investigate within the human population whether stimulant treatment for ADHD is associated with adult substance abuse. One reason is that ADHD, itself, is believed to be a risk factor that predisposes people to develop alcohol and other drug problems (Lynskey & Hall, 2001). Evidence supporting a role for ADHD in substance abuse comes from studies reporting elevated rates of childhood ADHD among people seeking treatment for opiate, cocaine, and other substance
abuse disorders (Carroll & Rounsaville, 1993). In one study, 35% of 298 people who sought treatment for cocaine abuse met the criteria for childhood ADHD (Carroll & Rounsaville, 1993). Those who reported childhood ADHD were younger at presentation for treatment and reported earlier onset of cocaine abuse, more frequent and intense cocaine use, higher rates of alcoholism, and higher rates of previous treatment than those who did not report ADHD. In a longitudinal study, a sample of children referred to treatment for problems of inattention and hyperactivity were later assessed at age 18 (Mannuzza, Klein, Bessler, Malloy & LaPadula, 1998). As part of this study, subjects with ADHD were compared to a matched control group who did not meet the criteria for ADHD. Those with ADHD were at a heightened risk for antisocial personality disorder and non-alcohol substance abuse disorder.

Another confounding variable in examining the relationship between stimulant treatment for ADHD and substance abuse is the co-morbidity between ADHD and other behavioral disorders. There is a large body of literature showing a high degree of correlation between attentional difficulties and conduct problems (Lynskey & Hall, 2001). One study reported that nearly 90% of a sample of 128
clinic-referred children diagnosed with ADHD also met lifetime criteria for co-morbid conduct or oppositional disorder (Biederman, Wilens, Mick, Spencer & Faraone, 1999). Children who develop conduct disorders are at heightened risks for a broad range of adverse outcomes in adolescence and later life, including increased rates of substance use and substance use-related problems. The linkages between early attentional difficulties and later substance use may therefore reflect the associations between ADHD and conduct disorder and those between conduct disorder and later substance abuse (Lynskey & Hall, 2001).

Despite the difficulties presented to researchers, a limited amount of research has investigated the relationship between stimulant treatment for ADHD and substance abuse. The research in this area has produced controversial results (Biederman et al., 1999; Lambert & Hartsough, 1998; Levin, Evans, McDowell & Kleber, 1998). Some research suggests that stimulant treatment decreases the risk of developing a substance abuse disorder while other research suggests that stimulant treatment increases the risk of developing a substance abuse disorder. One study supporting the theory that stimulant treatment reduces the risk of drug abuse reported that
methylphenidate treatment for ADHD not only reduced ADHD symptoms but also cocaine use in a sample of individuals with co-morbid ADHD and cocaine dependence (Levin et al., 1998). Subjects reported reductions in attention difficulties, hyperactivity, and impulsivity. In addition, self-reported cocaine use and craving decreased significantly. These findings have been supported by results of a study of substance abuse in young people with ADHD (Biederman et al., 1999). In the study, the cumulative incidence of substance abuse disorders between medicated subjects with ADHD, non-medicated subjects with ADHD and children without ADHD were followed-up for a period of 4 years. Those subjects with ADHD who received medication were much less likely than non-medicated ADHD subjects to develop a substance abuse disorder. Specifically, medication was associated with an 85% reduction in risk of developing a substance abuse disorder. Additionally, those subjects who received medication were at approximately the same risk for developing a substance abuse disorder as the non-ADHD controls.

One study obtained results that support the theory that stimulant treatment for ADHD increases the risk of substance abuse (Lambert & Hartsough, 1998). In the study,
subjects were divided into three subgroups. One was comprised of ADHD subjects who had received treatment with methylphenidate. A second group of subjects had been diagnosed with ADHD, but had not received treatment with methylphenidate or other CNS stimulants. A third group was comprised of age-matched controls who did not have ADHD. Frequency of lifetime cocaine use was measured. The medicated ADHD subjects showed the highest percentage of cocaine abuse, as indicated by DSM-III-R diagnosis, double that of either the non-medicated subjects or the age-matched controls. In addition, the study examined differential rates of adult smoking among the ADHD subjects with different stimulant medication histories. It was found that those ADHD subjects who had used stimulant medication for a year or more had a significantly higher rate of daily smoking than subjects who had no history of stimulant medication. As evidenced by research using human subjects, it is still unclear as to whether stimulant treatment for ADHD increases or decreases the risk of substance abuse. In addition, interpretation of this research is challenging since it is difficult to factor out the variation between the medicated and non-medicated ADHD subjects. Because of this, it is important that animal
studies examining the relationship between stimulant treatment and substance abuse be conducted.
CHAPTER TWO

NEUROTOXICITY OF
AMPHETAMINES

Neurotoxic Potential of Methylphenidate

Most evidence supports the use of amphetamine-like stimulants, particularly methylphenidate, as the best available pharmacotherapy in the treatment of Attention-deficit hyperactivity disorder (ADHD). While the therapeutic effects of methylphenidate are well-documented, little is known about the possible neurotoxic consequences of exposure to the drug. In particular, the question remains whether methylphenidate, like other amphetamines, produces toxic effects on brain monoamine systems. Methylphenidate acts similarly to other amphetamines in that it enhances dopaminergic transmission. Methylphenidate binds to the dopamine transporter and inhibits dopamine uptake with a potency similar to that of cocaine (Kuczenski & Segal, 1997). While the toxic effects of amphetamines have been known for quite some time, very few studies have examined the neurotoxic potential of methylphenidate. Those that did evaluate methylphenidate’s potential for neurotoxicity did not find
that it produced long-lasting changes in brain monoamine systems (Zaczek, Battaglia, Contrera, Culp & DeSouza, 1989; Yuan, McCann & Ricaurte, 1997). While the results of these studies suggest that methylphenidate lacks neurotoxic potential, much evidence has been generated regarding the neurotoxic action of amphetamine and related drugs.

Evidence for Amphetamine Neurotoxicity

Studies have revealed several markers of amphetamine-induced neurotoxicity on dopamine and serotonin systems. One such marker is a reduction in striatal tyrosine hydroxylase activity. Tyrosine hydroxylase plays a major role in the synthesis of dopamine. It is the rate-limiting enzyme in the production of L-DOPA, the precursor to dopamine. Methamphetamine administration has been found to decrease tyrosine hydroxylase activity in rat striatum (Koda & Gibb, 1973).

In addition to reducing striatal tyrosine hydroxylase, an abundance of research has shown that amphetamine causes long-lasting depletions of dopamine and its metabolites in the striatum (Abekawa, Ohmori & Koyama, 1994; Cass, 1997; Chapman, Hanson, Kesner & Keefe, 2001; Friedman, Castaneda & Hodge, 1998; O’Dell, Weihmuller & Marshall, 1991;
Repeated high doses of methamphetamine have been found to produce long-term depletions of dopamine in rat brain (Ricaurte et al., 1980). In addition, repeated administration of methamphetamine results in marked decreases in extracellular concentrations of dopamine metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in rat striatum (Abekawa et al., 1994; Ricaurte et al., 1982).

Additional evidence demonstrating the neurotoxicity of amphetamines comes from numerous studies that have shown that amphetamine reduces number of dopamine uptake sites (Jonsson & Nwanze, 1982; Nwanze & Jonsson, 1981; Steranka & Sanders-Bush, 1980; Wagner, Ricaurte, Johanson, Schuster & Seiden, 1980; Wagner, Ricaurte, Seiden, Schuster, Miller & Westley, 1980). In one study, the effect of methamphetamine on number of dopamine uptake sites in the striatum was examined (Wagner et al., 1980). Results showed that rats treated with methamphetamine had significant decreases in total number of dopamine uptake sites ($V_{max}$) as compared to saline treated rats. The affinity of residual sites ($K_m$) of methamphetamine treated
and saline treated rats was not significantly different. This indicates that reduction in dopamine is caused by a decrease in dopamine uptake site number and not a decrease in uptake site affinity. Additional research has shown that amphetamine, in addition to methamphetamine, produces a loss of dopamine uptake sites in the striatum (Wagner, Ricaurte, Johanson et al., 1980). The results of these two studies suggest that amphetamine and its analogs have toxic interactions with dopaminergic neurons resulting in long-lasting alterations of the dopaminergic system. These results are consistent with the hypothesis that there is dopamine terminal loss after high doses of methamphetamine treatment.

In addition to many studies indicating that amphetamines exert long-lasting neurotoxic effects on dopamine neurons, there is also anatomical evidence that dopamine nerve terminal degeneration follows amphetamine administration. Degeneration of nerve terminals has been found in the striatum of methamphetamine-treated rats (Ricaurte et al., 1982). There was, however, no evidence that dopamine cell bodies that give rise to these terminals were destroyed. This indicates that the toxic effect of methamphetamine is largely, if not exclusively, on dopamine
nerve terminals. In another study, to determine if this terminal degeneration induced by amphetamine was dopaminergic, the long-lasting dopamine depletion produced by methamphetamine was blocked with alpha-methyl-para-tyrosine (αMPT) (Ricaurte, Seiden & Schuster, 1984). No evidence of terminal degeneration was found in the striatum of any of the rats that were administered methamphetamine in combination with αMPT. The fact that preventing dopamine synthesis and depleting dopamine blocks degeneration induced by methamphetamine administration provides evidence that the degeneration is dopaminergic.

Lastly, methamphetamine treatment has been found to result in swollen axons in the striatum (Lorenz, 1980). Swollen axons in the striatum are indicative of increased neurotransmitter content. Other neurotoxins such as 6-hydroxydopamine (6-OHDA) also produce swollen, distorted axons (Malmfors & Sachs, 1968). The present morphological findings suggest that methamphetamine damages dopamine nerve terminals selectively in the striatum.

Free Radicals Theory of Amphetamine Neurotoxicity

Methamphetamine and amphetamine are selectively neurotoxic to dopamine and serotonin nerve terminals in the
central nervous system (Seiden & Sabol, 1996). The mechanism by which the neurotoxic effect of the amphetamine analogs occurs has been the subject of research for many years. Although several mechanisms have been proposed to explain dopamine-mediated neurotoxicity of amphetamines, current research supports the role of free radicals. In this model, it is proposed that amphetamine displaces dopamine from synaptic vesicles to the cytoplasm, allowing intraneuronal oxidative stress (Wan et al., 2000). This occurs because amphetamines cause an increase in dopamine levels that result in a higher rate of dopamine metabolism. Dopamine metabolism produces free radicals which are chemical species that contain unpaired electrons. They are highly reactive and can cause damage to nucleic acids, lipids, and proteins. Once a free radical is formed, it can react with dopamine to form 6-OHDA, a toxic hydroxy radical. It is postulated that with large amounts of dopamine in the synaptic cleft after methamphetamine treatment, a small proportion of dopamine could be metabolized to 6-OHDA and transported back into the dopamine neuron through the dopamine transporter (Seiden & Sabol, 1996). This model suggests that the formation of toxic hydroxy radicals from dopamine is the specific
mechanism by which amphetamines exert their neurotoxic effect.

Evidence for the role of free radicals in the neurotoxicity of amphetamines comes from research showing that administration of antioxidants such as ascorbic acid or vitamin E attenuates methamphetamine-induced neurotoxicity (DeVito & Wagner, 1989; Wagner et al., 1985), whereas inhibition of the antioxidant, superoxide dismutase (SOD), by diethyl-dithiocarbamate increases neurotoxicity (DeVito & Wagner, 1989). Research has shown that there is a production of superoxide radicals in the striatum of mice treated with methamphetamine (Cadet, Ladenheim, Baum, Carlson, and Epstein, 1994; Hirata, Ladenheim, Carlson, Epstein, and Cadet, 1996). This increase in superoxide radicals is caused by the release of dopamine after methamphetamine administration and subsequent dopamine oxidation within dopamine terminals (Baldwin et al., 1993; Marshall et al., 1993). Studies such as these have led to the theory that toxicity of methamphetamine and associated increase in dopamine oxidation is the result of free radical formation.
Dopamine Is Essential for Neurotoxicity

Evidence has shown that dopamine is essential for methamphetamine-induced neurotoxicity (Gibb & Kogan, 1979; Schmidt, Ritter, Sonsalla, Hanson & Gibb, 1985). When αMPT, which blocks dopamine synthesis, is administered concurrently with methamphetamine, it prevents methamphetamine-induced decreases in tyrosine hydroxylase activity and on concentrations of dopamine and its metabolites (Gibb, Johnson & Hanson, 1990). When dopamine synthesis was reinstated by concurrent administration of L-DOPA, a precursor to dopamine, neurotoxicity was again observed (Gibb & Kogan, 1979).

Additional evidence supporting the role of dopamine in methamphetamine-induced neurotoxicity comes from research with dopamine antagonists such as haloperidol and chlorpromazine. The effect of methamphetamine on both tyrosine hydroxylase and on concentrations of dopamine and its metabolites were completely blocked by administration of chlorpromazine (Buening & Gibb, 1974). These results provide evidence that dopamine mediates methamphetamine-induced neurotoxicity.
Conclusion

Research has shown that amphetamines produce long-lasting neurochemical and morphological alterations to the dopaminergic system. Because of this, amphetamines are considered neurotoxic to dopamine nerve cells. Specifically, amphetamines have been found to exert their neurotoxic effect by reducing striatal tyrosine hydroxylase activity, depleting striatal dopamine and its metabolites, decreasing number of dopamine uptake sites, and resulting in dopamine nerve terminal degeneration. The dopaminergic system has been implicated in several important functions, including movement, attention, learning, and the reinforcing effects of drugs of abuse (Carlson, 2001). Therefore, the neurotoxic effects produced by amphetamines may disrupt the overall functioning of the dopaminergic system. One particular area of interest is whether amphetamines alter brain neurochemistry to the extent that drugs of abuse become more rewarding. Furthermore, the question remains whether amphetamine-induced neurotoxicity increases vulnerability towards drugs of abuse.
CHAPTER THREE
DEVELOPMENTAL DIFFERENCES IN
AMPHETAMINE NEUROTOXICITY

Resistance to Methamphetamine Neurotoxicity in Young Rats

Methamphetamine administration in adult rats results in neurotoxicity characterized by reductions of neostriatal dopamine, tyrosine hydroxylase, and dopamine transporter sites (Bowyer et al., 1993). In addition to effects on the dopamine system, methamphetamine administration induces increases in striatal glial fibrillary acidic protein (GFAP) in adults (Pu & Vorhees, 1993). While adult rats exhibit a characteristic pattern of long-term neurotoxic effects when administered methamphetamine, developing rats appear to be resistant to methamphetamine-induced neurotoxicity (Cappon et al., 1997). Methamphetamine administered on PND 7-10 or PND 17-20 results in dopamine reductions only about half as large as those seen in adults (Lucot, Wagner, Schuster & Lewis, 1982). In addition, it has been found that there is a transition in susceptibility to methamphetamine-induced neurotoxicity that occurs around PND 40 in the rat (Pu & Vorhees, 1993). When examined 3
days following methamphetamine administration, PND 20 and PND 40 rats failed to demonstrate decreased tyrosine hydroxylase immunoreactivity. However, in rats dosed at PND 60, the normal adult pattern of decreased tyrosine hydroxylase immunoreactivity was observed. In addition, no increase in GFAP was found in methamphetamine-treated PND 20 rats, but a small increase was seen at 40 days. However, a large, adult-typical increase in GFAP was seen after day 60 of treatment. In another study, the acute and persistent monoaminergic responses of adolescent (PND 40) and young adults (PND 90) rats to multiple high doses of methamphetamine was compared (Kokoshka, Flickenstein, Wilkins & Hanson, 2000). Results showed that methamphetamine treatment significantly reduced dopamine transporter activity, tyrosine hydroxylase activity, and dopamine transporter ligand binding in the striatum of PND 90, but not PND 40 animals, to 33-53% of control values 7 days after treatment. These findings confirm previous findings that adolescent rats (PND 40) do not manifest long-term deficits in dopamine systems after exposure to methamphetamine treatment. From these studies, it is evident that there are age-dependent differences in the impact of methamphetamine on the dopamine system.
Hyperthermia in Methamphetamine Neurotoxicity

The severity of methamphetamine-induced neurotoxicity has been found to correlate with an accompanying thermoregulatory response, i.e., a hyperthermic response facilitates neurotoxicity while a hypothermic response is neuroprotective (Cappon et al., 1997). Depletion of striatal dopamine following methamphetamine administration is linked to a hyperthermic response (Bowyer et al., 1994). Specifically, decreases in striatal dopamine levels depend on the degree of hyperthermia produced during methamphetamine exposure. In addition, several agents that block dopamine depletions do so by inhibiting methamphetamine-induced hyperthermia. Haloperidol, diazepam, and dizoclipine (MK-801) all reduced methamphetamine-induced striatal dopamine depletion to a degree predicted by their inhibition of hyperthermia. Also, cold environments that blocked methamphetamine-induced hyperthermia also blocked methamphetamine neurotoxicity. In addition, when marked hyperthermia was produced, it was shown that methamphetamine increased the number of astrocytes that contained GFAP immunoreactivity and silver-degeneration staining in axons and terminals in
the striatum. From this study, it can be concluded that body temperature is a critical determinant of methamphetamine neurotoxicity.

Hyperthermia in Resistance to Methamphetamine Neurotoxicity in Young Rats

A thermoregulatory response has been proposed as a contributing factor in the resistance to methamphetamine neurotoxicity in developing rats. The thermoregulatory and neurotoxic effects of methamphetamine administration in developing rats at PND 20, PND 40 and PND 60 were investigated (Cappon, Morford & Vorhees, 1997). Rats at PND 20 and PND 40 were administered methamphetamine at ambient temperatures of 22°C and 30°C, and PND 60 rats were administered methamphetamine at 22°C only. Temperatures were measured and thermal responses were compared by calculating the total thermal response (TTR) induced by methamphetamine treatment. Results showed that methamphetamine administration to PND 60 rats at 22°C induced a hyperthermic response, resulted in a 47% reduction in neostriatal dopamine and a 49% increase in GFAP content. Administration of methamphetamine to PND 40 rats at 22°C failed to induce a hyperthermic response and
did not result in reduced dopamine or increased GFAP. However, administration of methamphetamine to PND 40 rats at 30°C induced hyperthermia, reduced neostriatal dopamine by 54% and increased GFAP by 70%. Methamphetamine administration to PND 20 rats at either 22° or 30°C did not result in dopamine depletion or increased GFAP, even though methamphetamine administration to PND 20 rats at 30°C induced hyperthermia. This study provides evidence confirming that the transition in neostriatal susceptibility to methamphetamine occurs at approximately 40 days of age. By contrast, the adult pattern is fully developed by PND 60. In addition, 20-day-old rats are resistant to dopaminergic and GFAP effects induced by methamphetamine treatment.

These findings illustrate the pivotal role of methamphetamine-induced hyperthermia in the ontogeny of methamphetamine-induced neurotoxicity (Cappon et al., 1997). Methamphetamine administered to PND 40 rats at 22°C failed to induce hyperthermia and did not result in dopamine depletion or reactive gliosis. This suggests that PND 40 rats are resistant to methamphetamine-induced neurotoxicity. However, PND 40 rats treated with methamphetamine at an ambient temperature of 30°C
demonstrate hyperthermia, dopamine depletion, and increased GFAP. Hence, at this age, resistance to methamphetamine-induced neurotoxicity may be overcome by elevating ambient temperature to produce hyperthermia. This implies that the neuroanatomical and/or neurochemical mechanisms underlying methamphetamine-induced neurotoxicity have developed by PND 40, but the components necessary for methamphetamine to induce hyperthermia are not yet fully mature. Another possibility is that PND 40 rats are able to dissipate excessive heat into the environment better than adults.

PND 20 rats administered methamphetamine at 30°C demonstrate a hyperthermic response comparable to that seen in PND 40 animals, yet they are resistant to methamphetamine-induced reactive gliosis and dopamine depletions. Consequently, PND 20 rats, unlike PND 40 rats, are resistant to methamphetamine-induced neurotoxicity despite induction of hyperthermia. This indicates that the neuroanatomical and/or neurochemical mechanisms responsible for methamphetamine-induced neurotoxicity are not present at PND 20. From this, it can be concluded that susceptibility to methamphetamine-induced neurotoxicity is dependent upon the developmental stage in which it is administered.
CHAPTER FOUR
ANIMAL MODELS OF DRUG REWARD

Introduction

Addiction and drug abuse are significant problems in the world today. Research on drug abuse and addiction takes place at many different levels. Within the field of neuropsychopharmacology, examination of the neural mechanisms of drug reward has become a major area of research. There are two measures that are most frequently used within a laboratory setting to assess the rewarding properties of drugs, conditioned place preference (CPP) and drug self-administration. With few exceptions, drugs that are self-administered have also been found to reliably produce CPP, and vice versa (Carr, Fibiger & Philips, 1989).

However, since the early 1980's, there has been some disagreement about whether CPP and self-administration represent two alternative methods for measuring a common reward process (Bardo & Bevins, 2000). The controversy has been fueled partly by different views regarding the nature of reward. Reward has sometimes been equated with the
subjective experience of pleasure, but science requires that the concept of reward be related to the organizing effects that it has on behavior (White, Messier & Carr, 1987). Two major organizing effects of reward have been identified (Carr et al., 1989). The most prominent effect is that stimuli which are generally agreed to be rewarding (incentive stimuli) have the capacity to elicit approach responses and maintenance of contact with stimuli (Schneirla, 1959). Another organizing effect of reward is its capacity to increase the probability that responses that precede it will be repeated. This strengthening of the association between environmental stimuli and preceding response has resulted in this effect being referred to as 'reinforcement' (Skinner, 1938).

Reward Versus Reinforcement

As noted in the behavioral sciences literature, it is important to remember that reinforcement and reward are two distinct concepts (White, 1989). Reinforcement is the process that occurs in the nervous system when contact with certain stimulus events (called reinforcers) produces a change in behavior (White et al., 1987). Skinner (1938) defined a reinforcer as an event that follows a response
and changes the probability that the response will be emitted in the future. A stimulus-response association may be strengthened by reinforcement, but this strengthening effect does not require that the reinforcer be rewarding or pleasurable. In fact, a reinforcer has the potential to be aversive or punishing. In a given environment, the presence of reinforcing stimuli serves to organize behavior by orienting the organism towards or against the stimulus object (Young, 1966). Therefore, reinforcement has come to refer to the tendency of certain stimuli to strengthen learned stimulus-response associations (White, 1989).

The notion of reward has its origin in the writings of philosophers, who described reward as individuals' natural tendency to maximize pleasure and minimize pain. This idea has not changed in any substantial way since it was first formulated. In modern psychology, the operationalization of affective states (e.g. reward and aversion), by Young (1959), provided the model used now for studying these behavioral processes. According to Young’s view, the operational definition of reward is approach; the operational definition of aversion is withdrawal. In the behavioral sciences, reward refers to the fact that certain environmental stimuli have the property of eliciting
approach responses and maintenance of contact. The most common contemporary measures of reward are electrical self-stimulation or self-administration of drugs, and various place preference techniques.

Self-Administration Paradigm

The self-administration paradigm is a valid and direct measure of the reinforcing properties of the drug (Koob & Goeders, 1989). The drug self-administration technique first requires a surgical procedure whereby a catheter is inserted into the jugular vein of the animal. This allows for the intravenous infusion of drug. The animal is then placed in an operant chamber for self-administration training and testing. The operant chamber is an enclosed environment with a small lever on the inside wall. A lever press will result in an intravenous injection of a drug. A signal light mounted above the lever can be used to indicate the onset of injection and remains lit for a period of time, during which the lever press will no longer result in drug infusion. Lever-pressing is reinforced under a schedule of reinforcement (Koob & Goeders, 1989). Training usually takes place under a fixed ratio (FR1) schedule of reinforcement. Under a FR1 schedule, every
lever press results in a drug infusion. Once regular lever-pressing has been established, the schedule of reinforcement can be increased. Through the process of operant conditioning, the animal will learn that drug infusion is contingent upon pressing the lever. The drug is considered to be reinforcing if it increases the probability of the lever-pressing response (Koob & Goeders, 1989). The experimental analysis of biological and environmental variables which modify the reinforcing efficacy of the drug, i.e., the extent to which a drug is self-administered, has implications for research on problems of human drug-seeking behavior, addiction, and dependence (Koob & Goeders, 1989).

Conditioned Place Preference Paradigm

CPP has been developed as an animal model of drug reward. To demonstrate CPP, animals are given a drug in association with distinct environmental cues. A typical CPP experiment includes differentially pairing two distinct sets of environmental (contextual) cues with the drug stimulus. The contextual cues tend to differ along several stimulus dimensions. The contexts may vary in flooring, size or shape, wall color or pattern, and olfactory cues.
Conditioning involves an animal receiving repeated exposure to the drug stimulus (termed unconditioned stimulus or US) in one context (termed conditioned stimulus or CS). Intermixed with these context-drug pairings is similar exposure to the other context without exposure to the drug US (Bardo & Bevins, 2000). Following conditioning is a choice test in which animals receive unrestricted access to both contexts in the absence of drug (US). Therefore, during testing, the animal is in a drug-free state. An increase in time spent in the paired context relative to time spent in the paired context prior to conditioning is taken as evidence that the drug (US) is rewarding (Bardo & Bevins, 2000). Presumably, after receiving pairings of a drug with a particular environment, an animal that is now drug-free will spend more time in the drug-paired environment than in the neutral environment. The increase in time is attributed to the conditioned reinforcing properties of environmental stimuli that have previously been paired with drug (Hoffman, 1989). The drug is said to be rewarding because the animal has shown a preference for the environment paired with drug as opposed to the neutral environment.
What Is Learned in Conditioned Place Preference?

CPP is based on principles and operations of classical (Pavlovian) conditioning. The CPP paradigm is based on the assumption that animals will approach rewarding stimuli and that neutral stimuli can acquire secondary rewarding properties through association with primary rewards (Carr et al., 1989). The primary rewarding properties of the drug serve as an unconditioned stimulus that is repeatedly paired with a previously neutral set of environmental stimuli. The neutral set of environmental stimuli acquire, in the course of conditioning, secondary rewarding properties. Having acquired secondary rewarding properties, they can act as conditioned stimuli which elicit approach when the animal is subsequently exposed to these stimuli in the absence of drug (Tzschentke, 1998).

Several lines of evidence support the assumption that CPP involves the acquisition of a reinforcing conditioned response (CR) in which reinforcing properties of the drug become associated with environmental stimuli (Bardo, Miller & Neisewander, 1984). First, it has been shown that various drugs may serve as effective primary reinforcers (Grabowski & Cherek, 1983). Second, environmental stimuli
which are paired reliably with a drug may elicit a conditioned response that mimics unconditioned drug effects. For example, a low dose of morphine produces hyperthermia, and stimuli associated with this drug effect can elicit a similar hyperthermic conditioned response (Miksic, Smith, Numan & Lal, 1975). Third, evidence indicates that environmental stimuli associated with a reinforcing drug can direct operant behavior. For example, rats injected with morphine in association with an environmental stimulus will perform an operant response which delivers the environmental stimulus alone (Schuster & Woods, 1968).

In CPP, the conditioned response (CR) is not observed directly, but rather is assumed to be reflected in the increased time that an animal spends in the presence of drug-associated stimuli (Bardo et al., 1984). This assumption is substantiated by research showing that the CPP paradigm demonstrates other principles of Pavlovian conditioning. In Pavlovian conditioning, extinction is a technique for producing a reduction and eventual disappearance of the conditioned response (Klein, 2002). Extinction involves repeatedly presenting the conditioned stimulus without the unconditioned stimulus (Mazur, 1999).
The strength of the conditioned response decreases as the number of CS-alone experiences increases until, eventually, the conditioned stimulus (CS) elicits no conditioned response (CR) (Klein, 2002). Research has successfully demonstrated that the CPP response (CR) can be extinguished when drug-associated stimuli are presented alone following conditioning (Mueller, Perdikaris & Stewart, 2002; Mueller & Stewart, 2000).

Another principle of Pavlovian conditioning that has been successfully demonstrated using the CPP paradigm is reinstatement. Reinstatement involves the ability of conditioned stimuli (CS) to once again elicit a conditioned response (CR) following extinction. Once a CPP (CR) has been extinguished, it can be reinstated following a priming injection of the drug (US) (Parker, & McDonald, 2000; Wang, Luo, Zhang & Han, 2000). The extinction / reinstatement paradigm is often used as a model of relapse to drug use (Fuchs, Tran-Nguyen, Specio, Groff & Neiswander, 1998).

Comparison Between Self-Administration and Conditioned Place Preference

There are three major advantages of drug self-administration. First, self-administration of a drug by an
animal is a direct measure of the reinforcing properties of the drug. The drug increases the probability of a response and thus acts as a reinforcer. This construct therefore enables the use of classical operant techniques for the measurement of motivational values of a drug, allows for the measure of relative reinforcement value of drugs, and controls for non-specificity of action of drugs and assessment of treatment effects (Koob & Goeders, 1989).

Another advantage of drug self-administration is that clear dose-effect functions can be obtained even in continuous reinforcement situations, and these dose-effect functions lend themselves to pharmacological antagonism. An antagonism results in a shift of the dose-effect function to the right, which at certain doses, is reflected in an actual increase in responding for drug. Injection of an antagonist would produce an increase in the number of self-injections of drug. This increase is generally considered to reflect a competitive functional interaction. The rat presumably increases drug self-administration to compensate for the decreased effectiveness of the drug as a reinforcer in the presence of partial receptor occupancy by the antagonist. Consequently, an increase in self-administration resulting from administration of a drug
antagonist is qualitatively similar to the effects of decreasing the dose of drug per injection (Koob & Goeders, 1989).

The third advantage of self-administration for the study of drug reinforcement is that this procedure can be used to study the biological site of action of drugs. Systemic and local intracerebral injections of pharmacological antagonists and neurochemically specific neurotoxins can be combined with these behavioral procedures to define site and mechanism of action for the reinforcing properties of drugs (Koob & Goeders, 1989).

There are three main disadvantages of drug self-administration as measures of the reinforcing properties of drugs. First, surgery is required that can be difficult in small rodents. Several particular precautionary procedures must be used to prevent contamination, and reduce the problems associated with blood clots and infection (Koob & Goeders, 1989).

Secondly, the lever-press response has to be learned, and time is required for animals to reach stable baselines. For heroin and cocaine, a 7-to-14-day period is required for animals to stabilize lever-pressing response (Koob & Goeders, 1989).
The final disadvantage centers on the general non-specificity of action of drugs. Drugs, rarely, if ever, have one specific neuropharmacological action, and in the intravenous technique, drug is delivered throughout the brain and body. This limits the interpretation of some of the behavioral preparations, particularly if 'non-reinforcing' actions are interfering with the ability of the animal to respond. Intracranial self-administration is one procedure that can be used to circumvent many of the non-specific effects associated with systemic drug delivery (Koob & Goeders, 1989).

The main advantages of CPP as an animal model of drug reward are (1) the CPP paradigm allows for either a preference (CPP) or an aversion (CPA) to be observed after drug conditioning. Thus, a major benefit in using this technique is that both rewarding as well as aversive properties of a drug can be determined using the same behavioral task; (2) time spent in the environment can be measured in an animal that is drug-free. Thus, any other effects of the drug that may coexist with its rewarding/aversive properties would not directly influence the time spent in the previously drug-paired environment. In contrast, using a self-administration paradigm, the
lever-pressing response is measured while the animal is under the influence of the drug. Therefore, the possibility exists that the lever pressing response may be confounded by the stimulating or sedating effects of the drug; (3) the CPP preference has been observed with relatively low doses of drug as compared to self-administration; (4) the possibility exists that CPP can be demonstrated in as little as one drug pairing, and when multiple pairings are used, these pairings can be conducted either once or twice daily without a decrement in the associative strength of conditioning. Thus, the relatively short time necessary for this procedure (i.e., it only requires 1 or 2 weeks) is an advantage. This contrasts with self-administration in which repeated self-infusions of drug are required in order to establish reliable lever-pressing behavior. This repeated exposure protocol likely affects receptor transduction mechanisms related to tolerance and/or sensitization (Bardo & Bevins, 2000); (5) CPP allows for simultaneous assessment of place preference and locomotor activity; (6) CPP typically yields dose effect curves that are monophasic. This contrasts with self-administration which typically yields a biphasic dose effect curve; (7) Drugs that have been shown to produce CPP
are consistently shown to be rewarding/reinforcing in other behavioral paradigms. Drugs of abuse are able to produce CPP and possess reinforcing properties in operant behavioral tasks (Carr et al., 1989). Thus, predictions from the CPP task as to the rewarding properties of drugs are consistent with predictions from other behavioral paradigms (Schechter & Calcagnotto, 1993).

Despite its numerous advantages, there are some limitations of CPP as a measure of drug reward. (1) There are some questions regarding what exactly is being measured by the CPP paradigm (Schechter & Calcagnotto, 1993). The possibility exists that animals are not really expressing a drug-environmental paired preference, but rather are simply being affected by locomotor activating / sedating actions of the drug. (2) A major concern regarding CPP is the potential confounding influence of novelty-seeking behavior on test day. It is well established that rats prefer a novel context over a familiar context (Hughes, 1968). This finding leads to the possibility that pairing drug with one context retards or blocks complete familiarization to that context, thus rendering it more novel relative to the saline context on the drug-free test day (Bardo & Bevins, 2000). One way that researchers have dealt with the issue
of novelty is to test animals in an apparatus that has three distinct contexts - one that is novel, one that is drug-paired, and one that is saline-paired. When tested in this situation, rats show a preference for the drug-paired context relative to the novel context (Parker, 1992). (3) Deficits in performance produced by drug-state dependency may be considered another limitation of CPP. Animals are trained in one state in that they experience the drug effect in a particular environment and they are later tested in a different state (drug-free). The possibility exists that the inability to express a preference for the drug-paired environment is inherent in the fact that training and testing occur in different subjective states (Schechter & Calcagnetti, 1993). (4) A final limitation of CPP is the difficulty in generating the type of dose-effect relationship normally expected in behavioral pharmacology (Bardo & Bevins, 2000). In many cases, does-response relationships have not been observed by varying the dose of drugs used successfully to produce a CPP. What is often observed is a "step-up" dose-effect relationship where one dose does not produce CPP but the next higher dose produces a positive and maximal effect (Schechter & Calcagnetti, 1993).
Are Conditioned Place Preference and Self-Administration Comparable Methods for Measuring Drug Reward?

There has been conflicting data as to whether CPP and drug self-administration measure a common reward process. Partial support for the claim that CPP is similar to self-administration arises when one compares the ability of each paradigm to detect reward across various drug classes. There appears to be reasonable concordance between drugs that produce CPP and drugs that are self-administered. Various stimulants, opiates and other drugs are known to support both CPP and self-administration (Bardo & Bevins, 2000). In contrast, neither CPP nor self-administration is produced by a host of other drug classes, including antagonists for dopamine, opioid, and cholinergic receptors, as well as antidepressants that work on either noradrenergic or serotonergic systems (Bardo & Bevins, 2000).

Despite this commonality, there are some significant exceptions to the general concordance between CPP and self-administration across drug classes. CPP may be unique in its ability to detect the rewarding effect of lysergic acid diethylamide (Meehan & Schecter, 1998), buspirone (Balster,
1990), and pentylenetetrazole (Gauvin, Dormer & Holloway, 1991), whereas self-administration may be unique in its ability to detect the rewarding effects of pentobarbital (Collins, Weeks, Cooper, Good & Russell, 1984) and phencyclidine (Marquis, Webb & Moreton, 1989). This discordance indicates that CPP and self-administration are not similar measures of a common reward process (Bardo & Bevins, 2000).

In addition to the discrepancy between CPP and self-administration noted across some drug types, several recent studies have demonstrated a clear dissociation between CPP and self-administration. In one study, rats were allowed to self-administer cocaine for either 6 or 29 sessions. These two groups were then examined for cocaine CPP, as well as reinstatement of self-administration using a cocaine cue (Deroche, Le Moal & Piazza, 1999). Although the 29-session group self-administered more cocaine than the 6-session group and showed greater sensitivity to cocaine’s ability to reinstate operant responding, no group differences in the dose response curve for cocaine CPP were obtained. These results provide strong evidence that CPP and self-administration are measuring fundamentally different processes.
There are also several examples which indicate that the neuropharmacological mechanisms that underlie CPP and self-administration are dissociable. One illustration of this point comes from studies examining the effects of D₂ dopamine antagonists on cocaine CPP and self-administration in rats. In general, studies have shown that systemic administration of cocaine induces a CPP that is not altered by pretreatment injections of various D₂ antagonists administered either systemically (Cervo & Samanin, 1995) or directly into the nucleus accumbens (Baker, Khroyan, O’Dell, Fuchs & Neisewander, 1996). This outcome contrasts with self-administration studies demonstrating that the reinforcing effect of cocaine is attenuated by D₂ antagonists administered either systemically (Ettenberg, Pettit, Bloom & Koob, 1982) or into the nucleus accumbens (Phillips, Howes, Whitelaw, Robbins & Everitt, 1994). Thus, D₂ dopamine receptors appear to be involved in the primary reinforcing effect of cocaine, but not in the rewarding effect of contextual stimuli paired with cocaine (Bardo & Bevins, 2000).

In conclusion, it is apparent that there is conflicting evidence as to whether CPP and self-administration are similar measures of drug reward.
However, the emphasis should not be placed on demonstrating CPP and self-administration as measures of a common reward process. Numerous studies have shown both paradigms to be valid measures of drug reward. CPP and self-administration uniquely contribute to our understanding of the neural mechanism underlying drug reward. Further research utilizing these two animal models of drug reward will ultimately add to our understanding of drug addiction and abuse liability.
CHAPTER FIVE
THE DOPAMINE SYSTEM
AND REWARD

Introduction

The dopaminergic system has been profoundly implicated in reward mechanisms. It is generally acknowledged that dopamine receptors in the mesolimbic system mediate the motivational-affective role of dopamine, while dopamine in the striatum is responsible for facilitating complex motor responses (Ikemoto & Panksepp, 1999). The mesolimbic system includes the dopamine pathway projecting from the ventral tegmental area (VTA) to the nucleus accumbens (Carlson, 2001). The nucleus accumbens is often cited as a structure linked with the reward role of dopamine (Stellar, Keley & Corbett, 1983). The nucleus accumbens can be divided into two major sub-regions: the shell (ventromedial part) and the core (dorsolateral part) (Ikemoto & Panksepp, 1999).

Self-Administration Studies

The most convincing evidence that supports the role of the mesolimbic dopamine system in reward is that animals self-administer chemicals that mimic dopamine (i.e. direct
dopamine receptor agonists) or increase extracellular dopamine (i.e. indirect agonists, e.g., amphetamine) directly into the nucleus accumbens. In operant procedures, the response contingent delivery of dopamine agonists directly into the nucleus accumbens can serve as a reinforcer for that response (i.e. increase its future probability of occurrence or strength of association) (Ikemoto & Panksepp, 1999). Rats self-administer d-amphetamine within the nucleus accumbens more so than equal amounts of saline (Hoebel, Monaco, Hernandez, Aulisi, Stanley & Lenard, 1983). In addition, rats will self-administer nomifensine, a dopamine reuptake blocker, into the nucleus accumbens (Carlezon, Devine & Wise, 1995). These results suggest that response-contingent dopamine reuptake blockade within the nucleus accumbens is sufficient to establish and maintain instrumental response habits. Furthermore, rats acquire and maintain self-administration of direct dopamine receptor agonists, a mixture of SKF 38393 (a D1-like agonist) and quinpirole (a D2-like agonist), into the nucleus accumbens (Ikemoto, Glazier, Murphy & McBride, 1997).

Additional support for the role of dopamine in reward comes from research on substance abuse. Mammalian species
readily acquire and maintain self-administration of psychostimulants such as amphetamine and cocaine (Schuster & Thompson, 1969). Amphetamine and cocaine are known to stimulate dopamine release and block reuptake in the nucleus accumbens. The mesoaccumbens dopamine system appears to play a critical role in mediating reinforcing effects of these psychostimulants. The role of dopamine in the reinforcing properties of psychostimulants can best be exemplified by studies showing that pharmacological manipulation of the dopamine system can affect self-administration of these drugs. In one study, dopamine nerve terminals in the nucleus accumbens were destroyed with 6-OHDA (Lyness, Friedle & Moore, 1979). It was found that 6-OHDA injections into the nucleus accumbens abolished the acquisition of d-amphetamine self-administration. The results suggest that dopamine neurons in the nucleus accumbens subserve the activation of a reward system. Removal of dopaminergic nerve terminals would result in a failure of indirect agonists like d-amphetamine to produce a positive (rewarding) stimulus. Therefore, one would expect poor acquisition of self-administration behavior. Additionally, in an effort to define the neural circuitry involved in the reinforcing effects of cocaine, the effect
of 6-OHDA-induced lesions of specific catecholamine pathways and terminal areas on cocaine self-administration was examined (Roberts, Koob, Klonoff & Fibiger, 1979). After cocaine self-administration behavior had stabilized, the nucleus accumbens was injected with 6-OHDA. When tested the day following the 6-OHDA injection, rats failed to self-administer cocaine. However, this disruption did not resemble extinction. After several days, self-administration recovered in many animals to near preoperative levels. The rate of this recovery correlated with levels of dopamine remaining in the nucleus accumbens. The animals with the greatest depletion of dopamine did not recover cocaine intake. The results therefore support the hypothesis that cocaine interacts with dopaminergic neuronal mechanisms to produce reinforcement and maintain self-administration behavior.

Conditioned Place Preference Studies

CPP has also been used to demonstrate the role of dopamine in the rewarding effects of d-amphetamine and direct dopamine agonists (Ikemoto & Panksepp, 1999). Intra-accumbens injections of amphetamine result in a strong CPP (Carr and White, 1986). The observation of a
CPP indicates that intra-accumbens injections were rewarding. Adding the dopamine receptor blocker cis-flupenthixol to the injection fluid attenuates the CPP produced by intra-accumbens amphetamine (Ausili & Hoebel, 1983). In addition, systemic injected haloperidol, a dopamine receptor antagonist, blocks amphetamine-induced CPP (Phillips, Spyraki & Fibiger, 1982). Dopaminergic involvement is also implicated by the attenuation of amphetamine CPP using selective 6-OHDA lesions of accumbens dopamine neurons (Spyraki et al., 1982). These findings suggest that dopamine release and receptor activation in the nucleus accumbens are both necessary and sufficient to produce CPP (White, Packard & Hiroi, 1991).

Brain Self-Stimulation Studies

The brain-stimulation reward paradigm has played a major role in initiating the idea of dopamine being a central substrate of brain reward (Lippa, Antelman, Fisher & Canfield, 1973). Animals readily learn to lever-press when the lever press results in a brief electrical stimulation (Olds, 1958). Electrical stimulation to the medial forebrain bundle, a major pathway interconnecting
the midbrain and forebrain, has been found to be strongly rewarding (Colle & Wise, 1988).

Systematic manipulations of brain dopamine receptors have marked effects on self-stimulation behavior. The most compelling evidence suggesting dopaminergic involvement derives from pharmacological studies. Dopamine agonists and antagonists, respectively, facilitate and disrupt self-stimulation behavior (Ikemoto & Panksepp, 1999). The effect of pimozide, a specific blocker of dopamine receptors, and amphetamine, a dopamine agonist, on self-stimulation of the medial forebrain bundle was examined (Gallistel & Karras, 1983). It was found that pimozide decreased rate of self-stimulation behavior, thereby decreasing the rewarding value of brain-stimulation. Conversely, amphetamine increased rate of self-stimulation, thereby increasing the rewarding value of brain-stimulation. Finally, the effect of concurrent administration of pimozide and amphetamine cancelled each other out. When administering the combination of pimozide and amphetamine, rate of self-stimulation was intermediate to the effects of the two individual treatments. These results support the hypothesis that pimozide and amphetamine exert their effects on reward via the same set
of dopaminergic synapses. In addition, application of brain stimulation in the VTA increases dopamine levels within the nucleus accumbens (Blaha & Phillips, 1990). This finding is consistent with the notion of a dopaminergic substrate of brain stimulation reward at electrode sites in the VTA.

Other Drugs of Abuse
Stimulate Dopamine Release

The potential importance for the role of dopamine in the rewarding properties of drugs is further highlighted by findings that many other drugs of abuse, including ethanol, opioids, cannabis and nicotine, share the ability to selectively stimulate dopamine release within the mesolimbic system (Ikemoto & Panksepp, 1999). Intraparietal administration of ethanol stimulates dopamine release and DOPAC and HVA output in the nucleus accumbens, and although less, also in the caudate (Imperato & Di Chiara, 1986). In addition, intravenous administration of both heroin and delta-9-tetrahydrocannabinol, the active ingredient in cannabis, increase extracellular dopamine concentrations in the nucleus accumbens (Tanda, Pontieri & Di Chiara, 1997). Lastly, nicotine administration results in an increase in dopamine transmission in the nucleus
accumbens and caudate (Imperato, Mulas & Di Chiara, 1986). These findings suggest that the reinforcing properties of these drugs may be mediated by its action on the mesolimbic dopamine system.

Conclusion

In summary, there is a large body of evidence supporting the involvement of dopamine in the rewarding value of stimuli. Behavioral studies such as self-administration, CPP and electrical brain stimulation all link dopamine to reward. Also, studies examining drugs of abuse have shown that dopamine mediates the rewarding properties of drugs. Additionally, studies have found dopamine to play a role in the rewarding properties of natural reinforcers such as food intake (Weingarten & Watson, 1989), sucrose consumption (Muscat & Willner, 1989), water intake (Gilbert & Cooper, 1987), and temperature reinforcement (Ettenberg & Carlisle, 1985). These highly consistent lines of evidence affirm that dopamine plays an important role in the reward system.
CHAPTER SIX

AMPHETAMINE IMPACTS REWARD

Introduction

It is now well accepted that amphetamine administration can be neurotoxic to dopamine neurons. A possible consequence of this toxicity is an alteration to neural reward centers as dopamine is known to be an important mediator of rewarded behavior. In addition, researchers have compiled compelling evidence that has implicated dopamine as a central substrate of reward (Lippa, Antelman, Fisher & Canfield, 1973). Since amphetamines are neurotoxic to the dopamine system, and being that dopamine plays an important role in reward, it is probable that amphetamine would impact reward mechanisms in the brain.

In an effort to investigate the impact of amphetamine on reward, researchers have conducted studies where they pre-treat animals with amphetamine and then assess rewarding effects of drugs using behavioral measures such as drug self-administration and CPP. However, there have been relatively few studies that have investigated the impact of repeated amphetamine exposure on reward.
Furthermore, the research that has examined the impact of amphetamine exposure on reward has been controversial. Some research indicates that amphetamines increase the rewarding value of drugs of abuse while other studies suggest that amphetamines decrease the rewarding value of drugs of abuse.

Behavioral Research: Evidence that Amphetamine Impacts Reward

One study obtained results that support the theory that prior exposure to amphetamine decreases the rewarding value of drugs (Itzhak & Ali, 2002). Specifically, this study examined the consequences of methamphetamine-induced neurotoxicity on methamphetamine place preference. The acquisition and reinstatement of methamphetamine-induced CPP was measured in drug pretreated and saline pretreated mice. Saline pretreated mice showed a much stronger preference for the methamphetamine-paired room as compared to the methamphetamine pretreated mice. Moreover, it took longer to extinguish the conditioned response of the saline pretreated group in comparison to the methamphetamine group. In addition, the saline pretreated rats showed stronger reinstatement of the place preference after a
priming injection of methamphetamine. The results of this study suggest that pretreatment with a neurotoxic dose of methamphetamine decreases the rewarding value of subsequent methamphetamine exposure. The results of this study support the theory that amphetamines decrease the rewarding value of drugs of abuse.

A similar study to that of Itzhak and Ali (2002) was conducted, but obtained results that support the theory that amphetamine pre-exposure increases the rewarding value of drugs (Gehrke, Harrod, Cass & Bardo, 2003). Rats were pretreated with a neurotoxic regimen of methamphetamine or saline, and were then conditioned 7 days later with methamphetamine (0.1, 0.3, or 1.0 mg/kg, s.c.) or saline using a CPP procedure. Results indicated that a neurotoxic dose of methamphetamine did alter subsequent methamphetamine CPP. For saline controls, only rats that received 1.0 mg/kg methamphetamine during conditioning demonstrated CPP. In contrast, rats that received the neurotoxic dose of methamphetamine demonstrated CPP when administered either 0.3 or 1.0 mg/kg methamphetamine. These results indicate that the rewarding effect of methamphetamine was enhanced by neurotoxic doses of methamphetamine.
Another study lends further support to the theory that amphetamines increase the rewarding value of drugs (Lett, 1989). Animals were divided into groups and pretreated with either d-amphetamine or morphine. Animals were then tested for either amphetamine-induced or morphine-induced CPP. Results showed that repeated exposure to amphetamine or morphine enhanced the drug-induced rewarding effects measured by CPP. This study also showed that cross-drug effects exist: exposure to amphetamine enhanced the rewarding effect of morphine, and exposure to morphine enhanced the rewarding effect of amphetamine. This cross-drug effect is of particular importance because it suggests that a common neural mechanism mediates the rewarding effect of drugs with varying mechanisms of action. The study suggests that with repeated exposure to drugs, the probability of addiction increases because drug-taking produces a progressively greater reinforcing effect each time it occurs (Lett, 1989).
The Dopamine D₂ Receptor Hypothesis: The Role of the Dopamine D₂ Receptor in the Ability of Amphetamine to Impact Reward

The neural mechanisms underlying the way in which amphetamine impacts reward is not completely understood, but it is hypothesized that dopamine D₂ receptors play a key role. Current research has shown that exposure to amphetamine reduces the number of dopamine D₂ receptor binding sites (Bennett, Hollingsworth, Martin & Harp, 1998; Chen, Su, Huang & Hsieh, 1999; McCabe, Hanson, Dawson, Wamsley & Gibb, 1987; Volkow et al., 2001). Furthermore, research has demonstrated that dopamine D₂ receptor levels are predictive of the rewarding properties of drugs of abuse. Studies have shown that lower levels of D₂ receptors are associated with increased drug-liking, pleasure, and euphoria (Laruelle, Abi-Dargham, van Dyck, Rosenblatt, Zea-Ponce & Zoghbi, 1995; Volkow, Wang, Fowler, Logan, Gatley, Gifford et al. 1999).

Evidence that Amphetamine Alters Dopamine D₂ Receptors

In one study, methamphetamine-induced reductions of D₂ receptors in several areas of the rat central nervous
system were measured (McCabe, Hanson, Dawson, Wamsley & Gibb, 1987). Results showed a reduction in amount of $[^3$H]sulpiride binding sites in rat striatum after multiple doses of methamphetamine. A significant reduction in $[^3$H]sulpiride binding was demonstrated in the striatum (29.4%), nucleus accumbens (36.7%), and olfactory tubercle (39.4%). Analysis indicated that the methamphetamine-induced reduction in $[^3$H]sulpiride binding to D$_2$ receptor sites was due to changes in number of receptors rather than affinity. These results suggest that receptor changes may be attributed to amount of dopamine released; methamphetamine releases amounts of dopamine that would result in a down-regulation of dopamine receptors. In another study, the effect of methamphetamine exposure on dopamine D$_2$ receptor binding in cultured midbrain dopamine neurons was examined (Bennett, Hollingsworth, Martin & Harp, 1998). Pretreatment with methamphetamine was found to decrease the B$_{\text{max}}$ for $[^3$H]raclopride binding, suggesting that methamphetamine causes a down-regulation of dopamine D$_2$ receptors. Additionally, following examination of D$_2$ receptor binding in rat striatum treated with amphetamine, it was found that the B$_{\text{max}}$ value of D$_2$ receptors in the striatum decreased 40% on day 7 and 52% on day 10 after
amphetamine withdrawal, without changes in their binding affinities (K<sub>d</sub>) (Chen, Su, Huang and Hsieh, 1999). The findings demonstrate that amphetamine administration for a period of 14 days leads to diminished D<sub>2</sub> receptor binding in the striatum at late withdrawal periods.

Research linking amphetamine to decreased dopamine D<sub>2</sub> receptor sites was extended by measuring levels of dopamine D<sub>2</sub> receptors in human methamphetamine abusers (Volkow et al., 2001). Fifteen methamphetamine abusers and 20 nondrug-abusing comparison subjects were studied using positron emission tomography (PET) to assess availability of dopamine D<sub>2</sub> receptors. Results indicated that methamphetamine abusers had a significantly lower level of D<sub>2</sub> receptor availability than comparison subjects (a difference of 16% in the caudate and 10% in the putamen).

The results of these studies suggest that amphetamine, in addition to causing changes in presynaptic dopamine markers, also reduce postsynaptic dopamine D<sub>2</sub> receptors. It was hypothesized that lower levels of D<sub>2</sub> receptors in amphetamine-treated subjects reflect receptor down-regulation from exposure to a higher extracellular dopamine concentration (Chen et al., 1998). The down-regulation of
D₂ receptors might reflect cellular mechanisms possibly associated with amphetamine-induced neurotoxicity

Dopamine D₂ Receptor Levels Predict Reinforcing Response to Drugs

Treatment with amphetamine results in a reduction in number of dopamine D₂ receptor binding sites. This finding is important, since studies have also demonstrated a relationship between drug addiction and number of dopamine D₂ receptors (Laruelle et al., 1995; Volkow et al., 1999). Specifically, these studies have found that number of dopamine D₂ receptors was predictive of the rewarding properties of psychostimulants.

In one study, the relationship between the behavioral effects of d-amphetamine and dopamine D₂ receptor levels was examined (Laruelle et al., 1995). SPECT imaging was used to measure dopamine D₂ receptor levels. Human subjects were injected with d-amphetamine, and the behavioral effects of the drug were measured by self-rating on the following analog scales: euphoria, alertness, restlessness and anxiety. Results showed that d-amphetamine injection induced a decrease in D₂ receptor availability. The d-amphetamine injection induced marked increase in euphoria,
alertness and restlessness scores. The intensity of these behavioral responses correlated with the decrease in D_2 availability measured with SPECT. In contrast, the anxiety response was milder and not correlated with decrease in D_2 availability.

In another study, PET was used to determine if there were differences in striatal dopamine D_2 receptor levels between those human subjects who reported the effects of methylphenidate as pleasant and those who reported them as unpleasant (Volkow et al., 1999). The study also assessed whether dopamine D_2 receptor levels predict behavioral responses to methylphenidate. Results indicated that subjects who liked the effects of methylphenidate had significantly lower dopamine D_2 receptor levels than subjects who disliked its effects. Moreover, the higher the dopamine D_2 levels found, the more intense were methylphenidate's unpleasant effects.

The results of these studies provide evidence that dopamine D_2 receptor levels predict response to psychostimulants in humans. Additionally, these results suggest that low levels of dopamine D_2 receptors may contribute to psychostimulant abuse by increasing pleasurable effects of drugs. Studies using human subjects
support the same conclusions as those using animal subjects: dopamine D2 receptor levels mediate reinforcing responses to drugs of abuse. However, while studies using human subjects have shown dopamine D2 receptor levels to increase the reinforcing response to drugs, animal studies have shown dopamine D2 receptor levels to decrease the reinforcing response to drugs. This is evidenced by the decrease in the reinforcing effects of alcohol and morphine in mice that lack dopamine D2 receptors (Maldonado, Saiardi, Valverde, Samad, Roques & Borrelli, 1997; Phillips, Brown, Burkhart-Kasch, Wenger, Kelly & Rubinstein, 1998), and by the decrease in the reinforcing effects of cocaine in animals given drugs to block dopamine D2 receptors (DeWit & Wise, 1977; Spealman, 1990).

Conclusion

Research has demonstrated that amphetamines impact reward. While the neural mechanisms underlying the way in which amphetamine impacts reward is not clearly understood, it is hypothesized that the dopamine D2 receptor plays a role in the ability of amphetamines to affect the reward system. Amphetamine administration reduces number of dopamine D2 receptors. Moreover, number of dopamine D2
receptors is predictive of the rewarding properties of psychostimulants. Specifically, lower levels of dopamine D₂ receptor sites are associated with increased drug reward. However, research is needed to examine the role of D₂ receptor in the ability of amphetamine to impact reward. This research will expand our knowledge of the neurobiology of drug addiction and may help us understand why some individuals are more predisposed to abuse drugs than others.
CHAPTER SEVEN
EARLY AMPHETAMINE EXPOSURE
AFFECTS REWARD AND LATER
BEHAVIOR

Early Amphetamine Exposure
Affects Reward

There are very few studies that have directly investigated the impact of early amphetamine exposure on reward. The studies that have been done in this area have been exclusive to methylphenidate. This research has looked at the effect of early methylphenidate exposure on vulnerability towards drug abuse (Achat-Mendes, Anderson & Itzhak, 2003; Anderson, Arvanitogiannis, Pliakas, LeBlanc & Carlezon, 2001; Brandon, Marinelli, Baker & White, 2001). Some studies suggest that early methylphenidate exposure decreases later vulnerability towards drug abuse while other studies suggest early methylphenidate exposure increases later vulnerability toward drug abuse.

One study obtained results that support the theory that early methylphenidate exposure decreases later vulnerability towards drug abuse (Anderson et al., 2001). In this study, rats were given intraperitoneal injections of methylphenidate (2.0 mg/kg) or saline twice daily on PND
20 to PND 35. This period approximates pre-adolescence in humans. At PND 60, CPP was used to assess the rewarding effects of cocaine. Rats pretreated with saline during development showed dose-related increases in the amount of time spent in the environment associated with cocaine. In contrast, methylphenidate pretreated rats spent less time in environments associated with a moderate dose of cocaine. A higher dose of cocaine that established place preference in saline pretreated rats failed to establish place preferences in methylphenidate pretreated rats. The results of this study suggest methylphenidate exposure in developing rats decreases responsiveness to the rewarding effects of cocaine. The findings suggest that early exposure to methylphenidate makes drugs of abuse less rewarding later in life.

In contrast, another study obtained results suggesting that early methylphenidate exposure increases vulnerability towards drug abuse (Brandon et al., 2001). Adolescent rats were pretreated with either methylphenidate (2 mg/kg for 7 days) or saline on PND 35 to PND 42. In adulthood, the reinforcing effects of cocaine were measured using drug self-administration. It was found that adolescent rats pretreated with methylphenidate self-administered cocaine
in adulthood at a higher rate compared to rats pretreated with saline. It was concluded that adolescent exposure to low doses of methylphenidate may increase vulnerability to the reinforcing effects of cocaine. These findings support the theory that early methylphenidate exposure increases vulnerability towards drug abuse by increasing the rewarding value of drugs.

In another study that looked at the effect of adolescent methylphenidate exposure on drug reward, adolescent mice received intraperitoneal injections of methylphenidate (10 mg/kg) or saline on PND 26 to PND 32 (Achat-Mendes et al., 2003). In adulthood, induction and reinstatement of cocaine-induced CPP was assessed. Methylphenidate pretreatment during adolescence resulted in a reduced preference for the cocaine-paired compartment in adulthood as compared to the saline pretreated animals. However, 2 weeks following extinction of cocaine-induced CPP and withdrawal from cocaine, a priming injection of cocaine was given. The priming injection of cocaine reinstated significantly higher CPP in the methylphenidate group than the saline group. The findings suggest that exposing mice to methylphenidate during adolescence ultimately increases cocaine-induced reward in adulthood.
This increased reward was observed particularly after a 2-week withdrawal period from cocaine. It is suggested that exposure to methylphenidate in adolescence may cause enduring neural adaptations that impact the reward system in adulthood, causing a heightened propensity for drug relapse (Achat-Mendes et al., 2003). Methylphenidate may trigger long-lasting neural adaptations that are expressed as an increased sensitivity to a cocaine challenge following withdrawal from cocaine. This is believed to be relevant to vulnerability to drug relapse (Achat-Mendes et al., 2003).

Early Amphetamine Exposure
Affects Later Behavior

Although there are few studies that have directly investigated the effect of early amphetamine exposure on reward, there have been several studies that have examined the effect of early amphetamine exposure on other behavioral measures. While developing rats appear to be resistant to the neurotoxic effects of amphetamine, behavioral deficits following amphetamine exposure have been observed. Rats pretreated with methamphetamine during the preweanling period (PND 1-20) exhibit an augmented acoustic startle response, reduced locomotor activity, and
impaired performance in a complex multiple-T water maze (Vorhees, Ahrens, Acuff-Smith, Schilling & Fisher, 1994). Multiple-T mazes are considered to be tests of reference memory and appear to involve processes such as vector navigation using proximal cues (Etienne, 1992). Therefore, it appears that methamphetamine-pretreated preweanling rats have long-term deficits in memory processes that depend on stable cues and on determining spatial position based on time and direction of movement (Vorhees et al., 1994). In addition, methamphetamine pretreatment during the preweanling period results in adult learning deficits that are specific to spatial navigation and memory (Vorhees, Inman-Wood, Morford, Broening, Fukumara & Moran, 2000). Furthermore, methamphetamine pretreatment during the preweanling period induces selective impairment of reference memory-based spatial learning while sparing sequential cued, and working memory-based learning (Williams, Morford, Wood, Wallace, Fukumura, Broening & Vorhees, 2003). Taken together, this research suggests that the effects of methamphetamine exposure during the preweanling period are both long-lasting and stage dependent, impacting arousal as well as cognitive functions.
ADHD is the most common neuropsychiatric diagnosis in children. It is estimated that 3%-5% of children meet the diagnostic criteria for ADHD (Palfrey, Levine, Walker & Sullivan, 1985). Methylphenidate currently remains the single most effective way to treat ADHD. In addition, the clinical use of methylphenidate for 3- to 6- year old preschool children who meet the diagnostic criteria for ADHD is becoming more common. However, little is known about long-term effects of methylphenidate on the very young. Given the increasing number of young children being treated with methylphenidate, much more research is needed to compare the safety and efficacy of stimulant medication across development. It is important to consider the possibility of adverse effects of stimulant medication on the developing brain. One major concern is whether early methylphenidate treatment would alter the brain in such a way as to impact the rewarding value of drugs, thereby increasing later vulnerability towards drug abuse.

Amphetamines are known to be neurotoxic to the dopamine system, and dopamine systems play a critical role
in mediating the rewarding effects of drugs. Research has shown that amphetamine exposure can impact drug reward. However the results of this research have been controversial, as some research indicates that amphetamine exposure increases the rewarding value of drugs while other studies suggest amphetamine exposure decreases the rewarding value of drugs. The neural mechanisms by which amphetamines impact reward is not completely understood, but it is hypothesized that the dopamine D₂ receptor plays a key role. Repeated exposure to amphetamine reduces the number of dopamine D₂ receptor binding sites (Bennett et al., 1998; Chen et al., 1999; McCabe et al., 1987; Volkow et al., 2001). Current research has demonstrated that dopamine D₂ receptor levels are predictive of the rewarding properties of drugs (Volkow et al. 1999). Studies have shown that lower levels of D₂ receptors are associated with reports of increased drug-liking, pleasure, and euphoria (Laruelle et al. 1995).

Since methylphenidate is an amphetamine, the question remains as to whether repeated methylphenidate exposure, like other amphetamines, decreases dopamine D₂ receptor levels and impacts drug reward. It is possible that methylphenidate treatment during development may decrease
dopamine binding sites and thus increase later vulnerability towards drug abuse. A recent study found that dopamine D₂ receptor levels in the striatum declined as a function of methylphenidate therapy in patients with ADHD (Ilgin, Senol, Gucuyener, Gokcora, Atavci & Sener, 2001).

Research examining the effects of early methylphenidate exposure on vulnerability towards drug abuse is sparse, and the findings have been inconclusive. Some studies suggest that early methylphenidate exposure decreases later vulnerability towards drug abuse while other studies suggest early methylphenidate exposure increases later vulnerability toward drug abuse. Furthermore, there has not been any research that has examined the effect of methylphenidate exposure during the preweanling period on later vulnerability towards drug abuse. Research is needed to investigate the possible adverse consequences following methylphenidate treatment during the preweanling period. Of particular interest is the question of whether early methylphenidate treatment would increase later vulnerability towards drugs of abuse.

Therefore, for my thesis, I propose to determine whether treatment with methylphenidate during the preweanling period will alter the rewarding properties of
morphine in adult rats. To determine whether morphine is rewarding, I will use a CPP paradigm. In this paradigm, a rat is given morphine, and it is restricted to a particular compartment of the CPP apparatus. On alternating days, the rat is given saline, and it is restricted to a different compartment. The compartments of the CPP apparatus have distinctive colors and odors.

In the proposed study, I will investigate the effects of early methylphenidate exposure on the acquisition, extinction, and reinstatement of morphine-induced CPP. I predict that rats given early methylphenidate exposure will (1) spend a greater amount of time in the morphine-paired environment than the saline exposed rats, (2) that the morphine-induced place preference will be harder to extinguish in the methylphenidate pretreated rats than the saline pretreated rats, and (3) that the morphine-induced place preference will be more readily reinstated in the methylphenidate pretreated rats than the saline pretreated rats. In investigating the extinction and reinstatement of morphine-induced CPP, I will be able to determine the consequences of early methylphenidate exposure on morphine withdrawal and relapse. I propose that methylphenidate exposure during development causes long-lasting neural
alterations that have the potential to impact not only vulnerability towards drug abuse, but also, vulnerability towards drug relapse. Should the results of my experiment support my hypotheses, it will have implications for the clinical use of methylphenidate in young children diagnosed with ADHD.
CHAPTER NINE

METHODS

Subjects

Subjects were 83 male and female rats of Sprague-Dawley decent, born and raised at California State University, San Bernardino. Rats were kept with the dam until weaning, when they were placed into group cages with same-sex littermates. No more than one rat from each litter was placed into a particular group. The colony room was maintained at 21-23°C and kept under a 12-hour light/dark cycle.

Drugs

Methylphenidate hydrochloride was dissolved in saline and injected intraperitoneally (ip) at a volume of 5 ml/kg. Morphine sulfate was also dissolved in saline and injected subcutaneously (sc) at a volume of 1 ml/kg. Both drugs were purchased from Sigma Aldrich (St. Louis, MO).

In Vivo Drug Treatment

Starting at postnatal day (PND) 10, rats received daily injections of saline or methylphenidate (2 or 5 mg/kg, ip, 5 ml/kg). These daily injections continued for
10 consecutive days (this age span is analogous to early childhood in humans).

Conditioned Place Preference Apparatus

Conditioning and testing were done in a T-shaped chamber consisting of three compartments. The two large end compartments are adjacent to each other and separated by a removable partition. The two end compartments measure 24 × 30 × 45 cm. The small compartment (the placement chamber) projected out from the junction between the large compartments. The small compartment measured 24 × 10 × 45 cm. A second removable partition enabled rats to enter either of the large compartments from the placement chamber. The odor, flooring, and color of each compartment varied. One of the large end compartments had white walls, wire mesh flooring, and pine bedding. The other large compartment had black walls, metal rod flooring, and cedar bedding. The placement chamber had a solid wood floor and was painted gray.
Preconditioning Day

On Day 1, following acclimation to handling, a total of 83 (n = 6 per group) 60-day-old rats were put into the placement chamber of the apparatus. After rats entered either the black or white compartment, access to the placement chamber was blocked, and rats were allowed 15 min of free access to both large end compartments. Preferences for the black and white compartments were determined for each rat at the end of the day. No injections were given on the preconditioning day.

Conditioning Phase

There were 8 daily conditioning sessions (Days 2-9) lasting 30 min each. On each conditioning day, rats either (a) received an injection of morphine (1 or 5 mg/kg, sc, 1 ml/kg) and placed in their non-preferred compartment, or (b) received an injection of saline and placed in their preferred compartment. Injections of morphine and saline were alternated daily, and the initial drug order was counterbalanced between groups.
Test for Induction of Morphine-Induced Conditioned Place Preference

On the test day (Day 10), rats were put in the placement chamber, and given free access to the large end compartments for 15 min. The CPP procedure was videotaped, and time spent in each compartment was scored by experimenters who were blind to treatment conditions. Induction of CPP was determined by comparing total time spent in the non-preferred compartment with total time spent in the preferred compartment on Induction Test Day. Similar to the preconditioning day, rats received no injections on the test day.

Extinction Phase

For 8 consecutive days (Days 11-18), all rats were given daily injections of saline. Each rat underwent 4 saline sessions in the previously drug-paired compartment and 4 saline sessions in the previously saline-paired compartment. Placement in the previously drug-paired compartment and previously saline-paired compartment were alternated daily.
Test for Extinction of Morphine-Induced Conditioned Place Preference

On Day 19, a test for extinction was performed following the same procedure used in the testing for induction of CPP. Rats were put in the placement chamber, and given free access to the large end compartments for 15 min. Extinction of CPP was determined by comparing total time spent in the drug-paired compartment with total time spent in the saline-paired compartment on Extinction Test Day.

Test for Reinstatement of Morphine-Induced Conditioned Place Preference

On Day 20, rats received a priming injection (1 mg/kg) of morphine. Fifteen min later, rats were allowed free access to the two compartments for 30 min. The time spent in each compartment was recorded. Reinstatement of CPP was determined by comparing total time spent in the drug-paired compartment with total time spent in the saline-paired compartment on Reinstatement Test Day.
Experimental Design and Statistical Analysis

The study was a $2 \times 3 \times 2$ experimental design. Sex had 2 levels: male or female. Drug pretreatment had 3 levels: 0, 2, or 5 mg/kg methylphenidate. Drug treatment had 2 levels: 1 or 5 mg/kg morphine. Litter effects were controlled by treating litter as a random factor in all analyses.

**Body Weight**

Preweanling weights during the 10 days of methylphenidate drug administration were analyzed using a $2 \times 3 \times 10$ (day) between-subjects ANOVA. Adult weights on the 1st day of CPP testing were analyzed using a $2 \times 3$ between-subjects ANOVA. Tukey tests were used as a post hoc analysis to determine the differences between the 3 pretreatment groups.

**Preconditioning Compartment Preference**

Preconditioning room preference was analyzed using a $3 \times (\text{compartment})$ within-subjects ANOVA that compared total time spent in the black compartment with total time spent in the white compartment.
Induction of Conditioned Place Preference

Induction of CPP was analyzed using a 3 (pretreatment) x (post treatment) within-subjects ANOVA that compared total time spent in the drug-paired compartment with total time spent in the saline-paired compartment for each rat.

Extinction of Conditioned Place Preference

Extinction of CPP was analyzed using a 2 (sex) x 3 (pretreatment) x 2 (post treatment) between-subjects ANOVA that compared total time spent in the drug-paired compartment with total time spent in the saline-paired compartment between the different treatment groups. Tukey tests were used as a post hoc analysis to determine the differences between the 3 pretreatment groups.

Reinstatement of Conditioned Place Preference

Reinstatement of CPP was analyzed using a 2 (sex) x 3 (pretreatment) x 2 (post treatment) between-subjects ANOVA that compared total time spent in the drug-paired compartment with total time spent in the saline-paired compartment between the different treatment groups. Reinstatement was analyzed in 3 time blocks: 1st 15 min, 2nd 15 min and 30 min. Tukey tests were used as a post hoc
analysis to determine the differences between the 3 pretreatment groups.

**Locomotor Activity on the First and Last Day of Morphine Exposure**

Locomotor activity on day 1 and day 8 of conditioning was analyzed using separate 2 (sex) x 3 (pretreatment) x 2 (post treatment) between-subjects ANOVA. A 2 (sex) x 3 (pretreatment) x 2 (post treatment) x 2 (day) repeated measures analysis was also done to compare locomotor activity between the first and last day of drug exposure.
CHAPTER TEN

RESULTS

Preweanling Body Weights

Preweanling body weights were not differentially affected by methylphenidate pretreatment nor did they differ by sex. There was a significant increase in body weight from the first day of injections (PND 10) to the last day of injections (PND 19) with rats weighing significantly more with each additional day (see Figure 1) (Day main effect, $F(9,216)=1984.47$, $p < .01$, Tukey tests).

Adult Body Weights

At PND 61, males weighed significantly more than females regardless of drug pretreatment (Sex main effect, $F(1,24)=362.04$, $p < .01$). Interestingly, rats treated with 5 mg/kg methylphenidate were significantly heavier than rats treated with saline or 2 mg/kg methylphenidate. Further analyses, however, showed that this effect was only apparent in females (see Figure 2) (Sex x Pretreatment interaction, $F(2,24)=3.81$, $p < .05$, Tukey tests).
Rats were injected with methylphenidate (0, 2, or 5 mg/kg) from PND 10 to PND 19. Body weight was not differentially affected by methylphenidate pretreatment.

Figure 1. Mean Preweanling Body Weights During 10 Days of Methylphenidate Injections
Adult Weights

<table>
<thead>
<tr>
<th>Methylphenidate Pretreatment (mg/kg)</th>
<th>Body Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>300  ± a</td>
</tr>
<tr>
<td>2</td>
<td>200  ± a</td>
</tr>
<tr>
<td>5</td>
<td>200  ± ab</td>
</tr>
</tbody>
</table>

\( ^a \) Indicates a significant difference between male and female rats.

\( ^b \) Indicates a significant difference in female rats pretreated with 0 mg/kg methylphenidate.

Figure 2. Mean Body Weights (± SEM) on the First Day of Conditioned Place Preference Training (PND 61) for Male and Female Rats Previously Exposed to Methylphenidate (0, 2, or 5 mg/kg) from PND 10 to PND 19

Initial Chamber Preference

Regardless of drug pretreatment, rats showed an initial preference for the black compartment over the white.
compartment (see Figure 3) (Room main effect, $F(16, 40) = 2.19, p < .05$).

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Time Spent in CPP Compartments (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td></td>
</tr>
</tbody>
</table>

![Initial Preference Day](image)

Rats were previously exposed to methylphenidate (0, 2, or 5 mg/kg) from PND 10 to PND 19.

Indicates a significant difference from time spent in the white compartment.

Figure 3. Mean Time Spent in Each Compartment ($\pm$ SEM) on the Initial Preference Day (PND 60)

Induction of Conditioned Place Preference

Rats given either 1 mg/kg or 5 mg/kg morphine demonstrated induction of CPP, because rats spent more time
in the drug-paired room as compared to the saline-paired room (see Figure 4) (Room main effect, $F(1,17)=5.01, p < .05$). No significant sex or pretreatment effects were found.

![Acquisition Test Day](image)

Rats were given alternating daily injections of morphine (1 or 5 mg/kg) and saline on PND 61 to PND 68. Data for the drug-paired compartments were collapsed across the two doses of morphine.

* Indicates a significant difference from time spent in the saline-paired compartment.

Figure 4. Mean Time Spent in Each Compartment (± SEM) on the Induction Test Day (PND 68)
Extinction of Conditioned Place Preference

Methylphenidate pretreatment increased the time needed to extinguish CPP, as rats treated with 5 mg/kg methylphenidate were slower to extinguish than rats treated with the low dose of methylphenidate or saline (Pretreatment main effect, $F(2,3)=812.93$, $p < .01$, Tukey tests). Morphine dose also affected rate of CPP extinction, because rats treated with 1 mg/kg morphine were slower to extinguish than rats treated with 5 mg/kg morphine (Post treatment main effect, $F(1,3)=731.32$, $p < .01$). However, the ability of the high dose methylphenidate pretreatment (5 mg/kg) and the low dose morphine conditioning treatment (1 mg/kg) to increase time spent in the drug-paired room during extinction testing was only significant for female rats (see Figure 5) (Sex x Pretreatment x Post treatment interaction, $F(2,3)=237.84$, $p < .01$, Tukey tests). Overall, CPP was harder to extinguish in male rats ($M=196.629$ S, $SEM=44.484$) than in female rats ($M=180.804$ S, $SEM=39.140$) (Sex main effect, $F(1,3)=33.68$, $p < .05$).
During the first 15 min block on the reinstatement test day, CPP was more readily reinstated in rats pretreated with 5 mg/kg methylphenidate than rats pretreated with 2 mg/kg methylphenidate or saline (Pretreatment main effect, $F(2,16)=9.57$, $p < .01$, Tukey tests). In addition, there was a significant post treatment effect such that CPP was more readily reinstated in rats treated with 5 mg/kg morphine than rats treated with 1 mg/kg morphine (Post treatment main effect, $F(1,16)=7.11$, $p < .05$) (see Figure 6). There were no significant effects found in the second 15 min block of time. For the entire 30 min block of time, CPP was more easily reinstated in rats pretreated with 5 mg/kg methylphenidate ($M=1051.778 \pm 65.356$) than rats pretreated with 2 mg/kg methylphenidate ($M=747.000 \pm 78.351$) or saline ($M=890.621 \pm 81.186$) (Pretreatment main effect, $F(2,16)=4.29$, $p < .05$, Tukey tests). There were no significant sex effects found during reinstatement.
During extinction training (PND 69-PND 76), rats were injected with saline and given alternating daily placements in the black and white compartments.

\textsuperscript{a} Indicates a significant difference from rats pretreated with 2 mg/kg methylphenidate or 0 mg/kg.

\textsuperscript{b} Indicates a significant difference from rats treated with 5 mg/kg morphine.

Figure 5. Mean Time (± SEM) Spent in the Drug-paired Compartment on the Extinction Test Day (PND 77)
All rats were primed with a 1 mg/kg dose of morphine 15 min prior to testing.

a Indicates a significant difference from rats pretreated with 2 mg/kg methylphenidate or 0 mg/kg.

b Indicates a significant main effect from rats treated with 1 mg/kg morphine.

Figure 6. Mean Time (± SEM) Spent in the Drug-paired Compartment on the Reinstatement Test Day (PND 78)

Locomotor Activity

On the first day of morphine administration, rats treated with 1 mg/kg morphine displayed more locomotor activity than rats treated with 5 mg/kg morphine (Post treatment main effect, \( F(1,3)=18.28, p < .05 \)). Locomotor
activity did not differ between drug groups on the last day of morphine administration. Locomotor activity for rats treated with 5 mg/kg morphine increased significantly from the first drug exposure to the last drug exposure (see Figure 7) (Post treatment x Day interaction, \( F(1,3)=16.24, p < .05 \)). This sensitization effect was not found for rats treated with 1 mg/kg morphine. Neither methylphenidate pretreatment or sex affected locomotor activity of rats during the CPP procedure.
During conditioning, rats were injected with either 1 or 5 mg/kg morphine.

* Indicates a significant difference from rats treated with 5 mg/kg morphine on Day 1.

* Indicates a significant difference from rats treated with 5 mg/kg morphine on Day 8.

Figure 7. Mean Line Crosses (± SEM) on the First and Last Days of Conditioned Place Preference Conditioning.
CHAPTER ELEVEN

DISCUSSION

Effects of Preweanling Methylphenidate Exposure on Morphine-Induced Reward in Early Adulthood

Methylphenidate pretreatment has been found to significantly alter the rewarding properties of drugs in rats (Achat-Mendes et al., 2003; Anderson et al., 2002; Brandon et al., 2001). In the present study, it was found that methylphenidate exposure during the preweanling period affects morphine-induced CPP. Three original predictions were made regarding the effect of methylphenidate pretreatment on morphine-induced CPP. Specifically, it was hypothesized that: (1) rats given early methylphenidate exposure would spend a greater amount of time in the morphine-paired environment than saline-exposed rats; (2) the morphine-induced place preference would be harder to extinguish in methylphenidate-pretreated rats than saline-pretreated rats; and (3) the morphine-induced place preference would be more readily reinstated in methylphenidate-pretreated rats than saline-pretreated rats. The prediction that rats pretreated with methylphenidate would spend a greater amount of time in the
morphine-paired environment was not supported, because the induction of CPP did not differ among the methylphenidate pretreatment groups. It was also predicted that morphine-induced place preference would be slower to extinguish in methylphenidate-pretreated rats than saline-pretreated rats. This hypothesis was supported. Rats pretreated with the high dose of methylphenidate were slower to extinguish than rats pretreated with the low dose of methylphenidate or saline. Lastly, it was predicted that morphine-induced place preference would be more readily reinstated in methylphenidate-pretreated rats than saline-pretreated rats. This hypothesis was supported during the first 15 min block of time, because CPP was more readily reinstated in rats pretreated with the high dose of methylphenidate than rats pretreated with the low dose of methylphenidate or saline. The present results indicate that preweanling methylphenidate exposure has a long-term impact on the rewarding properties of morphine. Specifically, preweanling methylphenidate administration appears to increase the rewarding value of morphine. Past research supports the notion that early methylphenidate exposure impacts drug reward (Achat-Mendes et al., 2003; Anderson et al., 2002; Brandon et al., 2001). However, none of the
prior studies administered methylphenidate during the preweanling period. Rather, methylphenidate was administered during the pre-adolescent and adolescent stages of development. The reports examining the effects of adolescent methylphenidate exposure on drug reward are inconclusive (Achat-Mendes et al., 2003). For example, pretreating 4-week-old rats with methylphenidate (2 mg/kg) caused an increase in cocaine self-administration two weeks after methylphenidate treatment (Brandon et al., 2001). In contrast, pretreating rats with methylphenidate (2 mg/kg) on PND 20-35 reduced the rewarding effects of cocaine on PND 60 (Anderson et al., 2002).

Methylphenidate may impact drug reward differently depending on the developmental stage at which it is administered. Interestingly, exposure to methylphenidate during the adolescent period appears to increase drug responsiveness, while exposure during the earlier pre-adolescent period reduces responsiveness to later psychostimulant administration (Anderson et al., 2002; Brandon et al., 2001). The developmental stage at which methamphetamine is administered also impacts long-term neurotoxic effects, with developing rats failing to exhibit the characteristic pattern of long-term methamphetamine
neurotoxicity (Cappon et al., 1997). Therefore, it would not be surprising if the developmental stage during which amphetamines, such as methylphenidate, are administered has a differential effect on drug reward.

Body weight measurements provide additional evidence that methylphenidate's effects vary according to age. When administered on PND 10 - PND 20, methylphenidate and saline did not differentially affect body weight (See Figure 1). Not only did methylphenidate not inhibit weight gain during the preweanling period but on the first day of CPP testing (PND 61), females pretreated with the higher dose (5 mg/kg) of methylphenidate weighed more than females pretreated with the lower dose of methylphenidate (2 mg/kg) or saline. While this finding contradicts research showing that methylphenidate inhibits weight gain (Spencer et al., 1996; Vincent, Varley & Legger, 1990), most of these studies have administered methylphenidate during later stages of development. This indicates that there may be developmental differences in the way methylphenidate impacts body weight.

The results revealed a methylphenidate dose effect, with the higher dose of methylphenidate (5 mg/kg) causing an increase in the rewarding value of morphine. Rats
pretreated with the high dose of methylphenidate (5 mg/kg) were slower to extinguish as compared to rats pretreated with the low dose of methylphenidate (2 mg/kg). Similarly, CPP was more readily reinstated in rats pretreated with the high dose of methylphenidate than rats pretreated with the low dose. In fact, the low dose of methylphenidate had no effect on reward at all. This suggests that higher doses of methylphenidate are more likely to alter reward mechanisms in preweanling brain. However, rats pretreated with 2 mg/kg methylphenidate during adolescence self-administered cocaine at a higher rate than rats pretreated with saline (Brandon et al., 2001). This suggests that a low dose of methylphenidate does impact reward during later stages of development. It should be noted that while 5 mg/kg is considered a high therapeutic dose, young children are often prescribed a higher doses of methylphenidate than adolescents and adults (Volkow et al., 2001).

One of the major differences between the present study and previous studies is that all other studies assessed methylphenidate's impact on the rewarding properties of cocaine, while we examined morphine CPP. This has particular significance when discussing the role of the
mesolimbic dopamine system in reward. It is generally acknowledged that dopamine receptors in the mesolimbic system mediate reward (Ikemoto & Panksepp, 1999). Drugs of abuse share the ability to selectively stimulate dopamine release within the mesolimbic system (Ikemoto & Panksepp, 1999). Methylphenidate causes long-lasting neural alterations to the mesolimbic dopamine system, thereby impacting the rewarding properties of cocaine (Achat-Mendes et al., 2003; Anderson et al, 2002; Brandon et al., 2001). By demonstrating that methylphenidate exposure can also impact the rewarding properties of morphine, we provide further evidence that the mesolimbic dopamine system mediates reward. And more importantly, this provides further evidence that methylphenidate exposure causes neural alterations to the mesolimbic dopamine system.

The neural mechanisms underlying amphetamine’s ability to alter the mesolimbic dopamine system and impact reward are not completely understood. But I have hypothesized that dopamine D2 receptors play a key role. Exposure to amphetamine reduces the number of dopamine D2 receptor binding sites (Bennett et al., 1998; Chen et al., 1999; McCabe et al., 1987; Volkow et al., 2001). Furthermore, dopamine D2 receptor levels are predictive of the rewarding
properties of drugs of abuse. Lower levels of D
2 receptors are associated with increased drug-liking, pleasure, and euphoria (Laruelle et al., 1995; Volkow et al., 1999). Thus, it is possible that methylphenidate, like other amphetamines, decreases the number of dopamine D
2 receptor binding sites, with this effect being greatest at higher doses. This reduction in dopamine D
2 receptor binding sites may be responsible for an increase in the rewarding properties of drugs of abuse following methylphenidate exposure.

In the present study, methylphenidate impacted the extinction and reinstatement of morphine-induced CPP while not affecting induction. Interestingly, similar results were obtained when mice were pretreated with methylphenidate (10 mg/kg) from PND 26-32 and tested for the induction and reinstatement of cocaine-induced CPP (Achat-Mendes et al., 2003). While pre-exposure to methylphenidate initially impaired the acquisition of cocaine-induced CPP in adulthood, reinstatement of cocaine-induced CPP was greater in mice pretreated with methylphenidate. Taken together, these results suggest that methylphenidate-induced neural adaptations in the reward system are slow to develop. Ultimately, both
studies suggest that early methylphenidate exposure results in an enhanced response to the rewarding effects of drugs.

Implications for Use of Methylphenidate for the Treatment of Attention-Deficit/Hyperactivity Disorder in Early Childhood with Regard to Vulnerability Towards Drug Abuse

The present study indicates that preweanling methylphenidate exposure increases the rewarding properties of drugs of abuse. It has been found that methylphenidate exposure during the preweanling period causes enduring changes in the neurobiology of brain reward systems, thereby impacting drug reward. The preweanling period in rats is analogous to early childhood in humans. Therefore, our findings have implications for use of methylphenidate for the treatment of ADHD in early childhood. The age at which stimulant treatment is given seems to be an important factor, presumably because of the way it impacts the maturation of the dopamine system (Robbins, 2002). During early childhood, the brain dopamine system is still maturing (Solanto, 1998). Therefore, stimulant medication, such as methylphenidate, may affect the brain of a young child differently than the brain of an adolescent or adult.
Our findings suggest that preweanling methylphenidate exposure does impact the reward system, since it was found that methylphenidate administration increases the rewarding value of drugs of abuse. These findings imply that stimulant treatment during early childhood may act as a risk factor for drug-seeking and drug-taking in adulthood. Thus, one may be more susceptible to drug addiction because drugs of abuse become more rewarding following stimulant treatment in early childhood.

Our study found methylphenidate to impact extinction and reinstatement of morphine-induced CPP. The extinction/reinstatement paradigm is often used as a model of relapse (Fuchs et al., 1998). By using an extinction/reinstatement paradigm in the study, we were able to investigate the consequences of early methylphenidate exposure on morphine withdrawal and relapse. Our findings suggest that methylphenidate exposure during early childhood not only increases vulnerability towards drug abuse, but also increases vulnerability towards drug relapse. This suggests that the neural alterations triggered by methylphenidate exposure during early childhood are long-lasting and may potentially persist for many years.
Animal research assessing the effects of preweanling methylphenidate exposure on vulnerability towards drug abuse is sparse. Further research is needed to ascertain how neuroadaptations caused by stimulant exposure are affected by dose, age at exposure, and treatment duration (Anderson et al., 2001). In addition, further molecular work is needed to ascertain which neural substrates are responsible for methylphenidate’s impact on drug reward, with special attention given to measuring dopamine D₂ receptor levels. Given the findings of this study, it is important to consider the possibility of adverse effects of stimulant medication on the developing brain. There is a lack of research within the human population assessing the use of stimulant medication for preschool-aged children. This is particularly important because preschool-aged children diagnosed with ADHD may be treated with stimulants for many years (Connor, 2002). Our study suggests that particular attention should be given to further investigating the possibility that stimulant treatment during early childhood increases one’s vulnerability towards drug abuse. It is hoped that additional clinical research will examine the balance between the short-term
expediency of methylphenidate treatment and the longer-term risk of promoting future drug abuse (Robbins, 2002).
REFERENCES


124


