REPEATED AMPHETAMINE EXPOSURE AND THE ENDURING CONSEQUENCES ON BEHAVIORAL AND NEURAL PLASTICITY

BY

JESSICA J. STANIS

DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Neuroscience
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2013

Urbana, Illinois

Doctoral Committee:

Professor Neal J. Cohen, Chair
Associate Professor Joshua M. Gulley, Director of Research
Professor Susan L. Schantz
Assistant Professor Roberto Galvez

Abstract

Amphetamines (AMPH) are relatively unique amongst the category of psychoactive drugs, as they are both prescribed for therapeutic purposes at an alarmingly increasing rate and abused illicitly on account of their potential pleasurable effects (World Drug Report, 2012). In adult subjects, chronic AMPH use is associated with deficits in decision-making (i.e., impulsivity) and working memory (e.g., Bechara et al., 1994; McKetin and Mattick, 1998; Rogers et al., 1999; Ornstein et al., 2000). However, not as much research exists about adolescent drug exposure and the long-lasting consequences on cognitive function in adulthood. We hypothesize that drug-induced changes in brain function are a major contributing factor to the behaviors that characterize addiction. In addition, drug exposure during a maturational time, such as adolescence, could have delayed and harmful consequences well after treatment has ceased. Thus, a major goal of this thesis was to examine the enduring effects of repeated AMPH exposure on cortical function and cognitive behavior.

The experiments presented here were designed using methods very sensitive to prefrontal cortex (PFC) function. In experiment 1 (Chapter 5), single-unit recordings from actively behaving rats were used to examine the relationship between the locomotor response (i.e., the ability to induce sensitization) and medial PFC (mPFC) firing activity to repeated AMPH treatment. The results suggested that repeated AMPH led to very selective changes in mPFC function when behavioral sensitization was present. Specifically, the magnitude of excitatory responses increased with the development of sensitization but was less persistent with a maximal sensitized response. In experiment 2 (Chapter 2), the relationship between behavioral

sensitization and impulsive choice behavior (operant delay-discounting) was examined in rats that received prior, repeated AMPH treatment. Chronic exposure to AMPH did not cause rats to become more impulsive. However, long-lasting changes in AMPH-induced locomotor activity were observed. The results suggested that the neuroadaptations associated with behavioral sensitization did not cause enduring deficits in the aspects of cognition that the delay-discounting task requires. In experiment 3 (Chapter 3), we examined whether impulsivity might be a pre-existing trait by using an established model that is based on an initial drug response to a low dose of cocaine. The results suggested that differential sensitivity to cocaine-induced locomotion was predictive of impulsivity, and the potential differences could shed light into mechanisms contributing to the vulnerability for addiction.

In experiment 4 (Chapter 4), we took a multi-faceted approach to examine long-lasting consequences of chronic AMPH exposure on working memory function. We had three main factors in mind: method of drug exposure (repeated, intermittent versus escalation-binge), age of drug exposure (adolescence versus adulthood), and the initial sensitivity of a psychostimulant. While adolescent-treated rats were found to be less sensitive to the stereotypy-inducing effects of both the intermittent and escalation-binge methods than adult-treated rats, they were more vulnerable to exposure-induced learning and memory impairments as tested in adulthood. Interestingly, the age-related deficits agree with the equal, but opposite model of drug action. That is, the intermittent regimen impaired working memory in adolescent-treated and not adult-treated rats, whereas the escalation-binge regimen impaired working memory in adult-treated and not adolescent-treated rats. In addition, the delay dependent deficits in adolescent- and

adult-exposed rats were predicted based on their initial sensitivity to a single injection of cocaine.

In the experiments presented here, repeated exposure to AMPH was found to have long-lasting consequences on learning and memory processes. These deficits were associated with initial differences in cocaine-induced locomotor activity. Moreover, the deficits were dependent on both the age and method of drug exposure. Taken together, our findings suggest that particular populations of neurons are more vulnerable to AMPH-induced changes. This notion is key in interpreting findings that suggest sensitizing regimens of AMPH do not necessarily lead to long-lasting changes in behavioral tasks that are sensitive to changes in PFC function. Furthermore, focusing on pathway-specific changes, especially within a neurodevelopmental framework, will be key in future studies if we want to understand individual vulnerabilities to psychiatric disorders such as addiction.

To those who percolate with passion: may you inspire others with what you know, never be afraid to fail, and always drink great wine.

ACKNOWLEDGEMENTS

This project would not have been possible without the support of many people. First, many thanks go to my faculty mentors William T. Greenough, Joseph G. Malpeli, and Carol A. Barnes; your early guidance and encouraging support were greatly appreciated. Thank you, Joshua M. Gulley, for fostering my love of music and the confidence to succeed as a scientist, and making me a better writer. I would also like to thank my committee members for helpful feedback and support, and in particular, Neal J. Cohen for being such a positive influence in advising me to the finish line.

Behavioral work requires more than a great team. I owe many thanks to the dedicated undergraduates who volunteered their time in lab to significantly contribute: Eric (Rick) Ofman, Kiril Staikov, Alexander Krueger, Randi Burns, David Klein, Hector Marquez Avila, Martin White, Sean Conway, Acidalia Ortiz Rivera, Sergei Pourmal, Mayank Patel, Sara Ryoo, Scott Shipman, Colin Hu, Alex Chan, Jameese Sykes, Lauren Sehy, Ashley Light, Claire Berthold, and Chris DeFalco.

The funding that made this work possible came from multiple sources. I was fortunate to be awarded a two-year fellowship from the Developmental Psychobiology and Neurobiology Training Grant supported by the National Institute of Child Health and Human Development. Much of this work was also supported with grants awarded to Joshua M. Gulley by the National Institute on Drug Abuse.

And lastly, I would like to thank my husband Dan Horlander, parents, and friends, who endured this seemingly everlasting process with me, always offering love and support through this rewarding process.

TABLE OF CONTENTS

CHAPTER 1: GENERAL INTRODUCTION 1

CHAPTER 2: DISSOCIATION BETWEEN LONG-LASTING BEHAVIORAL SENSITIZATION TO AMPHETAMINE AND IMPULSIVE CHOICE IN RATS PERFORMING A DELAY-DISCOUNTING TASK 15

CHAPTER 3: DISPARATE COCAINE-INDUCED LOCOMOTION AS A PREDICTOR OF CHOICE BEHAVIOR IN RATS TRAINED IN A DELAY-DISCOUNTING TASK 34

CHAPTER 4: DIFFERENTIAL VULNERABILITY TO AMPHETAMINE-INDUCED DEFICITS IN A WORKING MEMORY TASK: THE EFFECTS OF EXPOSURE METHOD, AGE, AND INITIAL PSYCHOSTIMULANT SENSITIVITY 53

CHAPTER 5: ADAPTATIONS IN MEDIAL PREFRONTAL CORTEX FUNCTION ASSOCIATED WITH AMPHETAMINE-INDUCED BEHAVIORAL SENSITIZATION 78

CHAPTER 6: GENERAL DISCUSSION 101

CHAPTER 7: FIGURES AND TABLES 109

REFERENCES 132

CHAPTER 1: GENERAL INTRODUCTION

Historically, stimulants such as amphetamine (AMPH) were used to treat a number of medical ailments, and in particular, obesity, asthma, narcolepsy, and attention-deficit hyperactivity disorder (ADHD). Today, AMPH is widely accessible, in large part, because of the availability and illegal distribution of prescription stimulants, such as Adderall and Dexedrine (both amphetamines, different isomers). The prevalence of illicit use varies worldwide and it has been estimated that 33 million people have consumed amphetamines (World Drug Report, 2012), whereby individuals aged 12-24 report the highest rates of active use (estimated 19.5 million; Substance Abuse and Mental Health Services Administration, SAMHSA, 2011). Investigations into the motives behind illicit use in teenagers and young adults have revealed that most take amphetamines to enhance academic performance and alertness, as well as to produce euphoria (Teter et al., 2006; Judson and Langdon, 2009). As with other drugs of abuse, individuals can become dependent upon stimulant use. In fact, initiation of drug use before age 14 leads to approximately a four times greater likelihood to abuse or become dependent later in life (SAMHSA, 2006).

Cortical memory systems and drug abuse in humans

Much of the focus on neural systems and drug abuse has been directed at subcortical structures, such as the striatum (e.g., Graybiel et al., 1990; Robbins and Everitt, 1999; Everitt and Robbins, 2013). More recently, imaging evidence from human

substance abusers has strengthened a role for cortical and limbic systems as major structures adversely affected by chronic drug exposure. For instance, Rogers et al. (1999) found that AMPH abusers exhibited deficits in a decision-making task similar to those exhibited by patients with focused damage to their prefrontal cortex. Similarly, a study by Ornstein et al. (2000) tested subjects who were AMPH abusers on a test battery that examined a number of components that are sensitive to cortical damage, such as recognition memory, spatial working memory, planning, visual discrimination learning, and attentional set-shifting. While the authors failed to find impairments in tasks related to verbal fluency and recognition, they found a number of deficits in a majority of the decision-making and working memory tasks, supporting the hypothesis of frontal-executive dysfunction in chronic drug abusers. A more recent study by Ersche et al. (2008) showed that one form of response-reversal learning was impaired in chronic cocaine abusers, but not those abusing amphetamine. Careful attention should be given when interpreting data to prevent broad generalizations of learning and memory deficits related to the chronic use of different psychostimulants (also see Verdejo-Garcia et al., 2005). Furthermore, results from human studies make it rather difficult to ascertain whether co-morbid personality traits (e.g., excessive risk taking, sensation seeking, or impulsivity) and cognitive dysfunction predate the onset of drug use or occur as a consequence of chronic use. Current hypotheses suggest that chronic drug use leads to impairments in functions mediated by the prefrontal cortex and associated limbic circuitry (Jentsch and Taylor, 1999; Goldstein and Volkow, 2002; Koob and Volkow, 2010; Kalivas and Volkow, 2012). Thus, characterizing the extent of cognitive deficits associated with chronic psychostimulant exposure could provide

insight into underlying neural mechanisms of drug abuse and dependence. Alternatively, such impairments may have been pre-existent and intensified by subsequent drug exposure. Because the issue of causality has not been resolved in human studies, it is imperative that research in animal models can distinguish brain abnormalities that could predispose individuals to use drugs from changes that likely result from chronic drug abuse.

Cortical memory systems and drug exposure in animals

Preclinical (or animal) studies, including the experiments detailed here, offer the opportunity to distinguish hypotheses based on both predisposing factors and the interactions with chronic use of psychostimulants, such as AMPH. For example, long-term self-administration of cocaine in non-human primates has been associated with deficits in stimulus discrimination and delayed spatial alternation tasks that are sensitive to disruptions in PFC function (Liu et al., 2008). In rats allowed extended access to intravenous (i.v.) cocaine, but not those allowed limited access, impairments in working memory were observed (George et al., 2008). In rats exposed to 30 mg/kg cocaine for 14 days, impairments in reversal learning (Schoenbaum et al., 2004) and reinforcer devaluation (Scheoenbaum and Setlow, 2005) were reported. Deficits have also been reported following repeated AMPH exposure, particularly in tests of working memory (Castner et al., 2005), attentional set-shifting (Fletcher et al., 2005), and visual attention (Fletcher et al., 2007). In contrast to these results, a number of studies did not find impairments in working memory (Featherstone et al., 2008), spatial learning or memory (Russig et al., 2003), or delayed alternation (Stefani and Moghaddam, 2002) after

repeated exposure. Confounding variables that may contribute to these disparate findings among studies are the method of repeated drug exposure and the task specificities. However, this has not been analyzed systematically within the same study. Thus, a major aim of the experiments carried out here is to address how different drug exposure paradigms affect both locomotor and task behaviors that have varying levels of difficulty and more robust and thorough task components (i.e., learning and memory, strategy, reversal).

Repeated and intermittent drug exposure method

An influential animal model associated with repeated, intermittent administration of psychostimulants has been shown to induce long-lasting changes at the behavioral, anatomical, and neurochemical levels. This form of plasticity is referred to as behavioral sensitization (Robinson and Becker, 1986), or an increased response to the drug, that is evident in certain species-specific behaviors. For example, one would see motor hyper-responsiveness (i.e., increased locomotion and rearing) at relatively low doses and stereotypy, or patterns of repetitive behavior, at higher doses (Segal and Schuckit, 1983). Sensitization has also been described in humans, especially in the context of drug-induced psychosis (Robinson and Berridge, 1993; Bartlett et al., 1997; Ujike and Sato, 2004) and AMPH-induced euphoria, drug “liking”, and motor activation (Strakowski et al., 1996, 2001; Boileau et al., 2006). Furthermore, sensitization has been hypothesized to contribute to the development of compulsive behaviors that characterize addiction (Robinson and Berridge, 1993; Kalivas et al., 1998). An extensive literature suggests that the mesolimbic DA system, which includes cell bodies

in the substantia nigra and ventral tegmental area (VTA) that predominately innervate the dorsal striatum, NAc, and PFC, is a critical component of the neural circuitry that underlies behavioral sensitization.

Escalation and binge exposure method

Compared to intermittent drug exposure, the model of escalation and binge has been understudied in animals, even though human drug taking patterns are very different from those typically administered in an intermittent paradigm. In order to assess human-related deficits in cognition, it is especially important to simulate human abuse patterns in animal models. Humans commonly escalate doses of intake, after which the frequency of use increases (Gawin, 1991; Angrist, 1994; Gawin and Khalsa-Denison, 1996), resulting in high dose binge patterns of administration. It has been suggested that the previous period of escalation before binge episodes serves as a protectant to the neurotoxic or lethal effects associated with high acute stimulant doses (Seiden et al., 1975; Ricaurte et al., 1980; Fleckenstein et al., 2000; O'Dell and Marshall, 2000). Thus, the development of tolerance during escalation likely impacts subsequent drug-induced behavioral profiles differently than an intermittent, same dose paradigm. In fact, Segal and colleagues (2003) have shown that tolerance also develops to the stereotypy-inducing effects, which is paralleled with attenuation in the reduction of striatal DA content and DAT binding. This is in contrast with studies that show evidence for neurotoxicity and long-term declines in DA levels and plasma membrane DAT and the vesicular monoamine transporter (VMAT) in rats and from neuroimaging studies in humans (Wilson et al., 1996; Volkow et al., 2001; Chang et al.,

2007). While several studies have examined the effects of acute binge treatments on cognitive function and reported impairments in simple novel object recognition (NOR, Bisagno et al., 2002, 2003; Schroder et al., 2003), the escalating dose binge regimen has not been shown to cause impairments in that NOR task (Clark et al., 2007). Thus, it appears that dose escalation prior to the binge phase alters the behavioral and neurochemical responses in a way that might be more protective of long-term cognitive function. However, there have been no studies that have examined cognitive function using other tasks.

Initial sensitivity to psychostimulants

The possibility that individual differences underlying drug vulnerability might reflect distinct behavioral and neurobiological traits is supported in a number of human and animal studies. In humans, differences in the initial sensitivity to cocaine's effects are predictive of long-term use and dependence (Davidson et al., 1993; Haertzen et al., 1983; Schafer and Brown, 1991) and a recent study has indicated that individuals who reported a high degree of "liking" or "wanting" on their initial use of cocaine had a significantly increased risk of cocaine abuse (Lambert et al., 2006). In rodents, a reliable indicator of initial sensitivity to psychostimulants is drug-induced locomotor activity in an open-field arena. Cocaine, in particular (Briegleb et al., 2004) has variable stimulant effects on behavior, such that outbred rats can readily be classified as low or high cocaine responders (LCRs or HCRs, respectively) based on their response to a single treatment with cocaine (Sabeti et al., 2002; Gulley et al., 2003; Gulley, 2007; Klein and Gulley, 2009). Interestingly, these individual differences are related to

differences in the function of dopamine transporters (DATs) in the dorsal striatum and nucleus accumbens (Sabeti et al., 2002, 2003) and not to pharmacokinetic factors (Gulley et al., 2003). Subsequent studies have shown that the LCR/HCR phenotype can be used to predict a number of behaviors, such as conditioned place preference (Allen et al., 2007), intravenous cocaine self-administration (Mandt et al., 2008), and drug-discrimination (Klein and Gulley, 2009). Thus, at this point, we speculate that the overlap between phenotypic differences and subsequent behaviors can be related to the vulnerability for drug dependence. This is the basis for key experiments proposed here that extend these findings in relation to repeated psychostimulant exposure and subsequent interactions with ensuing behaviors from experimental paradigms that assess aspects of impulsivity and cognitive behavior.

Adolescent psychostimulant exposure and age-dependent changes

Most studies of the effects of repeated psychostimulant exposure have been carried out in adult subjects. However, adolescence, which in humans generally includes the ages of 12-18 years old, is a period when many individuals are first exposed to drugs of abuse. For some, initial use develops into long-lasting problems with drug abuse and addiction. In fact, studies have revealed that the use of psychoactive substances increases during adolescence, with a peak reached at age 18-20 (SAMHSA, 2011). Given this link between adolescent-onset of drug use and addiction, it is important to determine how early drug exposure affects an individual's susceptibility to develop drug abuse in adulthood.

A particularly advantageous experimental approach is to use rodent models of adolescence, partly because the developmental period of adolescence spans roughly two weeks (P35-50), rather than six or more years in humans (Spear, 2000). This offers the promise of a more ready determination of causal relationships between neural function and behavior. Importantly, the transition to adulthood in rodents entails many aspects found in human transition to adulthood, e.g., physiological maturation as well as ethological factors, such as departing from the early home environment and changing social companions. Adolescent rats also spend a large amount of time in social interaction and play behavior (Panksepp, 1981) and are hyperactive in exploring novel environments (Spear and Brake, 1983). The mesocorticolimbic dopamine (DA) circuitry is important for these behaviors, is also known to be critical for the locomotor-stimulating and reinforcing effects of psychostimulant drugs (Wise and Bozarth, 1987; Koob, 1992; Bardo, 1998), and is undergoing normal changes during the adolescent period (see Brenhouse and Andersen, 2011).

The underlying neuroadaptations in adolescents taking drugs are intriguing because the brain (and frontal cortex, in particular) is already in a state of development flux (see Casey et al., 2005; Brenhouse and Andersen, 2011). On a cellular level, the most prominent changes include: alterations in cell number and dendritic morphology (Cunningham et al., 2002; Markham et al., 2007), as well as synaptic pruning, with substantial synapse elimination of glutamatergic input to the PFC (Huttenlocher, 1984; Insel et al., 1990) and increases in dopaminergic inputs to the PFC (Kalsbeek et al., 1988; Leslie et al., 1991; Rosenberg and Lewis, 1994). Drugs are likely to perturb this

process in a way that has long-lasting implications that might not become evident until adulthood.

Unfortunately, we do not have a great understanding of the enduring consequences of early psychostimulant exposure. Several studies have shown that adolescent rodents are less sensitive to the acute effects of psychostimulants compared to adults on tasks that measure arousal, motivation, and rewarding properties of drugs; such tasks include locomotor activity, conditioned place preference, and preference for natural rewards (Laviola et al., 1995; Carlezon et al., 2003; Bolanos et al., 2003, respectively). Very few studies, though, have examined the long-term effects as expressed during adulthood. A study by Brandon et al. (2001) examined the effects that repeated methylphenidate (Ritalin) had on cocaine self-administration in adult rats. They found that adolescent exposure to methylphenidate facilitated the acquisition of cocaine self-administration in adulthood. Their findings suggest that those rats displayed an enhanced sensitivity to the rewarding properties of drugs. Several more studies have recently emerged regarding the effects of early psychostimulant exposure on cognitive function into adulthood. For example, deficits were found in object discrimination (Santucci and Rabidou, 2011) and stimulus-reward learning (Harvey et al., 2009) after repeated cocaine exposure, in spatial learning after repeated methamphetamine (Vorhees et al., 2005), and in impulse control after repeated AMPH (Hankosky and Gulley, 2012) during the adolescent period. No studies have simultaneously examined the long-lasting effects of repeated AMPH exposure on both locomotor sensitization and more demanding forms of cognition, like working memory and reversal learning, in the same subjects.

Specific Aims

Aim 1: To examine the neural correlates associated with repeated AMPH exposure and behavioral sensitization.

There is strong evidence of PFC plasticity in animals exposed to repeated AMPH. Such long-lasting changes have been demonstrated morphologically in dendritic branching and spine density (Robinson and Kolb, 1999), as well as electrophysiological in mPFC neurons *in vitro* (Peterson et al, 2006), in anesthetized rats *in vivo* (Onn and Grace, 2000; Peterson et al., 2000; Trantham et al., 2002), and in awake rats (Homayoun and Moghaddam, 2006; Sun and Rebec, 2006). Given that sensitization and neuroadaptations in the PFC are thought to be significant aspects responsible for driving behavioral changes associated with addiction, it is important to determine if there is a distinct relationship between neuronal activity and the emergence of locomotor adaptations. In experiment 1, we used single-unit recording techniques to address the following question: Are there lasting changes in the response of mPFC neurons to AMPH when behavioral adaptations are evident? The results of this study, which have been published (Gulley and Stanis, 2010) and are reproduced in Chapter 5, suggest that repeated AMPH led to very selective changes in mPFC function when behavioral sensitization was evident. The magnitude of excitatory responses increased in parallel with sensitization, but were much less persistent following an AMPH challenge (when locomotor sensitization was most robust). The data imply that particular populations of neurons are more vulnerable to AMPH-induced plasticity, and focusing in this notion will be key in future recording studies.

Aim 2: To determine if repeated AMPH exposure leads to enduring changes in sensitization and cognitive function, such as decision-making, working memory, and reversal learning.

Neuroadaptations in the PFC are hypothesized to play an important role in the behavioral changes associated with repeated psychostimulant drug exposure. While such exposure is known to cause long-term changes in AMPH-induced locomotor behavior (i.e., sensitization), it is not clear, however, if repeated AMPH exposure produces long-term changes in cognitive behaviors that, in humans, are hypothesized to contribute to addiction. In experiment 2, the following question was addressed: Does a repeated and intermittent AMPH exposure paradigm, which induces locomotor sensitization, alter decision-making? Rats were repeatedly treated with AMPH and then trained and tested in an operant task (delay-discounting) that is sensitive to their inability to tolerate longer delays for a more advantageous food reward. The results of this study, which have been published (Stanis et al., 2008b) and are reproduced in Chapter 2, suggest that the neuroadaptations known to be associated with locomotor sensitization do not overlap with those that lead to changes in impulsive choice behavior. In experiment 3, we asked whether impulsivity might instead be a pre-existing trait. The initial sensitivity to psychostimulants has been shown to predict their long-term use and dependence in humans. In rats, it has been shown that sensitivities to cocaine-induced locomotion are the result of differential cell-surface expression of the dopamine transporter (Briegleb et al., 2004), and the phenotype has been characterized on a number of other behavioral tasks in adult rats (e.g., Gulley et al., 2007; Allen et al., 2007; Mandt et al., 2008; Klein and Gulley, 2009). Experiment 3 addresses the

following question: Can we predict cognitive changes based on the initial sensitivity to the locomotor-activating effects a psychostimulant, such as cocaine? The results of this study, which have been published (Stanis et al., 2008a) and are reproduced in Chapter 3, suggest that differential sensitivity to cocaine-induced locomotion was predictive of impulsivity, and the potential differences could provide insight into the distinct neural mechanisms related to the vulnerability of addiction.

The final experiment was designed to address a number of questions that remained about long-lasting consequences of AMPH exposure and cognitive function. Because the enduring consequences of repeated AMPH exposure might be dependent on dose and duration of treatment, and we know that human drug taking patterns are very different from those typically administered in an intermittent paradigm, a regimen of AMPH on an escalation schedule was followed by high-dose binge administration. This model of escalation and binge has been understudied in animals, compared to the intermittent model. Thus, is AMPH treatment regimen- and task-specific in its ability to influence behavioral and cognitive changes? Moreover, adolescence in humans is a period of life when drug use, whether for therapeutic or recreational purposes, is often initiated. Unfortunately, it is also a time when individuals develop long-lasting patterns of drug abuse and addictive behaviors. Human and animal studies have revealed that during adolescence, brain areas such as the PFC are undergoing significant, yet normal, changes in synaptic connectivity. These changes in synaptic organization have important implications and might interact with AMPH in a way that leads to harmful consequences. Does exposure to AMPH during adolescence induce long-term modifications in behavior and cognitive function that endure into adulthood? And based

on findings from experiment 3, is there also a predictable relationship between the initial sensitivity to cocaine-induced locomotion and more specific cognitive functions, namely working memory? The results from this study suggest that chronic AMPH caused enduring consequences on cognitive function in both adolescent- and adult-treated rats, as tested in adulthood well after drug exposure ceased. Importantly, the nature and timing of normal developmental changes interact with drug exposure in a method-dependent manner. In addition, the working memory deficits were predicted based on the initial sensitivity to cocaine.

Summary

The number of prescriptions for amphetamine has dramatically increased by 1600% over the last decade (Berman et al., 2009). This is of huge concern because the abuse of prescription drugs has also been on the rise, especially in adolescents. Given that drug exposure during this time could have drastic, long-term effects on the brain and behavior, more studies are needed to examine age-dependent plasticity to psychostimulants, such as AMPH. The experiments discussed here contribute new knowledge on the long-term consequences during adulthood from both adolescent- and adult-AMPH exposed animals. By revealing such consequences, we can better understand the underlying neural mechanisms to treat and perhaps even prevent abuse in the future. Nevertheless, these studies contained here are of utmost importance to tease apart long-term effects based on the method of AMPH exposure and interactions with levels of vulnerability, such as the initial sensitivity to locomotor-activating effects of a psychostimulant and the age of initial exposure. Furthermore,

understanding the extent of drug-induced plasticity is an important step in characterizing transient and enduring changes that are associated with drug abuse and/or dependence. Successful treatment options will likely need to take into account the individual spectrum of deficits observed.

CHAPTER 2: DISSOCIATION BETWEEN LONG-LASTING BEHAVIORAL SENSITIZATION TO AMPHETAMINE AND IMPULSIVE CHOICE IN RATS PERFORMING A DELAY-DISCOUNTING TASK[1]

Abstract

Rationale: Repeated amphetamine (AMPH) exposure is known to cause long-term changes in AMPH-induced locomotor behavior (i.e., sensitization) that are associated with similarly long-lasting changes in brain function. It is not clear, however, if such exposure produces long-lasting changes in a cognitive behavior that, in humans, is hypothesized to contribute to addiction. *Objectives:* To examine whether repeated AMPH exposure induces both locomotor sensitization and alters impulsive choice in a delay-discounting task. *Methods:* Adult, male Sprague-Dawley rats (n = 29) were pre-treated with 3.0 mg/kg AMPH or saline every other day for 20 days, and were then trained to lever press for small, immediately delivered food reinforcement or larger reinforcements delivered after delays. We subsequently assessed the effects of acute AMPH (0.1-2.0 mg/kg) on delay-discounting. Lastly, we tested for long-lasting effects of pre-treatment by giving an AMPH challenge (3.0 mg/kg) one week after the final delay-discounting session. *Results:* Repeated AMPH produced sensitization to the drug's stereotypy-inducing effects, but did not alter acquisition or baseline behavior in the delay-discounting task. Following acute AMPH, impulsive choice and other measures of delay-discounting were altered, but to a similar extent in both saline- and AMPH-

[1] This chapter appeared in its entirety in the journal Psychopharmacology as Stanis JJ, Marquez Avila H, White MD, Gulley JM (2008) Dissociation between long-lasting behavioral sensitization to amphetamine and impulsive choice in rats performing a delay-discounting task. 199(4):539-548. This article is reprinted with the permission of the publisher and is available from http://www.springerlink.com and using DOI: 10.1007/s00213-008-1182-z

pretreated groups. The AMPH challenge, given ~3 months after the last pretreatment injection, revealed sensitization was still evident. *Conclusions:* Our results suggest that one behavioral consequence of repeated AMPH exposure – sensitization – does not overlap with another potential outcome – increased impulsivity. Furthermore, the neuroadaptations known to be associated with sensitization may be somewhat distinct from those that lead to changes in impulsive choice.

Introduction

While the locomotor effects and cellular adaptations that result from repeated amphetamine (AMPH) exposure have been well characterized (e.g., Robinson and Becker 1986; Seiden et al. 1993; Robinson and Kolb 1997, 1999; Lu and Wolf 1999), the long-term consequences of such exposure on measures of cognitive function are not as well established. For instance, a number of clinical studies have demonstrated impairments in decision-making (Rogers et al. 1999; Ersche et al. 2006), concentration and attentional control (Deller and Sarter 1998; McKetin and Mattick 1998), and working memory (Ornstein et al. 2000). However, most of these impairments were observed shortly after cessation of drug administration (≤ three days), thereby leaving the possibility that they were influenced by acute withdrawal effects rather than more long-term, drug-induced neuroadaptations that might persist well beyond drug withdrawal. Also, the results observed in clinical studies may be influenced by the existence of polydrug abuse, varied duration and frequency of drug use and/or abstinence, and co-morbid psychiatric disorders (Khantzian 1985; Levin and Kleber 1995; Rosselli and Ardila 1996).

Recent studies using animal models, which offer an advantage of control over many of these variables, have provided further insights into the long-term effects of repeated AMPH exposure on cognition. For example, rats that exhibit behavioral sensitization have been shown to have deficits in tasks measuring attentional set-shifting (Fletcher et al. 2005), sustained visual attention (Fletcher et al. 2007), pre-pulse and latent inhibition (Tenn et al. 2003, 2005), and, in primates, working memory (Castner et al. 2005). Rats that self-administered AMPH over five different cycles of five days each, which were interspersed with a nine day drug-free period when they were tested in a five-choice serial reaction time task, exhibited deficits in visuospatial attention and no significant effects on perseverative (compulsive) or premature (impulsive) responding compared to yoked, saline-infused controls (Dalley et al. 2005). The changes in attentional performance recovered within five days of the withdrawal period, but reappeared following a two-month abstinence period and acute treatment with 0.8 mg/kg AMPH. Impulsive responding was attenuated by this dose of AMPH in the group of rats with AMPH self-administration experience.

Another task used to assess impulsive behavior in an operant reinforcement context is delay-discounting. In this paradigm, the apparent value of reward can be shifted by manipulating the time delay between a required response and the delivery of an expected reinforcement (Evenden and Ryan 1996). Specifically, small, immediately delivered reinforcements become more valuable than those that are large, but presented following a delay (Monterosso and Ainsle 1999). Thus, in this paradigm, choices of small reinforcements are taken to reflect impulsive behavior. Although increased impulsivity is considered to be characteristic of individuals who abuse drugs

(e.g., Jentsch and Taylor 1999; Coffey et al. 2003; Kirby and Petry 2004; Heil et al. 2006; Hoffman et al. 2006; Verdejo-Garcia et al. 2007), there have been relatively few studies that have directly addressed the role of repeated psychostimulant exposure in altering impulsive choice behavior. Those that have (e.g., Richards et al. 1999; Paine et al. 2003; Roesch et al. 2007; Simon et al. 2007) suggest that chronic treatment with methamphetamine or cocaine increases impulsivity. However, a recent study by Winstanley et al. (2007) showed that repeated cocaine treatment did not alter baseline levels of impulsive choice. While this study was extensive, details about cocaine-induced locomotor activity (e.g., whether or not behavioral sensitization was observed) were not reported.

In the current study, we examined whether one behavioral consequence of repeated AMPH exposure—behavioral sensitization—overlaps with another potential outcome—impulsive choice. Because the delay-discounting task requires rats to be well-trained before responding becomes stable, any observed effects of AMPH pretreatment on task behavior are not likely to be due to disruptions caused by short-term withdrawal effects. After 40 daily sessions in the task, we examined whether acute treatment with saline (vehicle) or AMPH (0.1-2.0 mg/kg) just prior to delay-discounting test sessions altered subsequent task behavior. Lastly, rats in both the saline and AMPH pretreatment groups were given a challenge injection of AMPH (3.0 mg/kg) to determine if long-lasting changes in locomotor activity were evident. This challenge test occurred approximately 3 months after the last injection in the open-field.

Methods

Animals. Male Sprague-Dawley rats (n = 32), bred in our animal facility from stock rats obtained from Harlan (Indianapolis, IN), were housed individually starting at ~2 months of age and were 3-4 months old (315-450 g) at the start of experiments. They were maintained on a 12:12 h light: dark cycle (lights on at 0800) with experimental sessions conducted between 0900 and 1800 h. Rats were handled five times for 15 min intervals prior to being used in experiments. With the exception of periods when rats were undergoing operant training and testing, food was available *ad libitum*. Water was always available *ad libitum*. All experimental procedures were approved by the IACUC at the University of Illinois, Urbana-Champaign and were consistent with the *Principles of Laboratory Animal Care* (NIH Publication no. 85-23).

Apparatus. Locomotor activity was measured in an open-field chamber that consisted of a transparent, Plexiglas box (40.6 x 40.6 x 40.6 cm) surrounded by photobeams (Coulbourn Instruments; Allentown, PA). Each apparatus was connected to a computer operating software (TruScan, v. 2.01; Coulbourn Instruments) that recorded all horizontal and vertical beam breaks (100 ms sampling rate). The horizontal beam breaks, or coordinate changes, were converted into ambulatory distance (cm). The chambers were individually contained inside sound-attenuating cubicles (76 x 80 x 63 cm). Each cubicle contained a speaker (76 mm dia.) fixed to one wall, two ceiling-mounted white lights (4 W) for dim illumination, and a ceiling-mounted camera between the two lights.

Operant behavior was monitored in standard operant conditioning chambers (Coulbourn Instruments). One wall of the chamber contained two retractable levers that were positioned on either side of a centrally located food trough. Infrared detectors

were used to monitor head entries into the food trough. White cue lights were located above each lever. A white houselight was located near the top of the chamber on the opposite wall.

Repeated saline or AMPH treatment. Prior to starting the 20-day, intermittent injection procedure, rats were first habituated to the open-field testing and injection procedures. Specifically, they were brought to the testing room and, following a 20-min acclimation period during which they remained in their home cages, were placed in the open-field arena for 30 min. They were then removed, injected with saline (1 ml/kg, i.p.), and returned to the arena for 60 min. On the next day (i.e., treatment day 1), the same procedure was repeated except half of the rats (n = 16) received saline and the remainder were given AMPH (3.0 mg/kg, i.p.; n = 16). Beginning on treatment day 2 and continuing every other day until treatment day 9, rats were brought to the laboratory, injected with saline or AMPH, and allowed to behave for 60 min in an acrylic tub (46 x 25 x 22 cm) lined with hardwood bedding. These tubs and bedding, which were identical to those used for the rat's home cage in the animal colony, were used as a means to provide multiple injection environments and thereby minimize context-dependent sensitization (Robinson and Becker 1986; Badiani and Robinson 2004). On treatment day 10, the open-field testing procedure used on treatment day 1 was repeated. Thus, rats received a total of 10 saline or AMPH injections over the course of 20 days, with automated (i.e., photobeam) activity measures obtained on the first and final injection days. The intermittent-dosing procedure was adapted from previous studies that showed impairments in object recognition memory (Bisagno et al. 2003; Belcher et al. 2006) and the development of behavioral sensitization (Robinson et al.

1985; Robinson and Becker 1986). Furthermore, pilot studies revealed that rats given AMPH using this procedure exhibited progressive increases in focused stereotypy that persisted for at least one month after treatment.

Delay-discounting behavior. Following their last test in the open-field arena, rats were placed on food restriction and maintained at 85% of their free-feeding weight. One week later, they were trained to respond on either of two independently available levers for a 45-mg food pellet (Bio-Serv; Frenchtown, NJ) on a fixed ratio schedule (FR1) of reinforcement during overnight sessions (2100 to 0900 hours). After they displayed approximately equal responding on both levers, rats were moved to the next training phase (1-h sessions, between 0900 and 1700 h). Trials began with levers retracted, and the food trough illuminated by a cue light. A nosepoke into the trough resulted in the extension of one randomly selected lever, with a subsequent lever press response reinforced by delivery of a food pellet. After 3-4 days of training at this stage, 14 saline pre-treated and 15 AMPH pre-treated rats began the final stage of training. Three rats (n = 2 and 1 from the saline and AMPH pre-treated groups, respectively) failed to complete the first two training stages within 15 sessions and were thus removed from the study. Two different cohorts of rats were trained on the task, with the second cohort responding for a different formulation of food pellet then the first (Bio-Serv products F0042 and F0021, respectively) at the end of training. This switch was made because of pellet feeder clogging problems that developed towards the end of the second cohort's training. Statistical analysis of behavioral performance within cohort two following this switch, and between cohorts one and two, revealed no significant change in task performance.

Training in the delay-discounting task was done in daily, 100 min sessions that consisted of five blocks of 12 trials. Each trial lasted 100 s and began with the illumination of the house light and cue light located in the food trough. Rats were required to nosepoke into the trough within 10 s, whereupon a single lever would be presented randomly and a response was reinforced with food pellet delivery. The amount of food delivered was pre-assigned to a lever, such that responses on one (e.g., left side lever) resulted in reinforcement that was large (four pellets) but delayed in delivery, and responses on the other (e.g., right side lever) resulted in an immediately delivered small reinforcement (one pellet). Assignment of reward magnitude to levers was counterbalanced across groups, but remained consistent for each rat. After a lever response, the house light turned off, the levers retracted, and the cue light above the lever was illuminated until food was delivered. If the rat either failed to nosepoke within 10 s or respond on the presented lever, the trial was recorded as an omission. On omission trials, the levers remained retracted and the chamber was returned to an intertrial interval (ITI) state until the beginning of the next trial. After completing or omitting the first two forced-choice trials (which served as exemplars for the each block), 10 free-choice trials were presented where both levers were extended. Delays for the delivery of large rewards increased with each block of trials (0, 10, 20, 40, and 60 seconds, respectively). Each animal participated in one session per day, for a minimum of 40 training sessions.

Following the last training session, the effects of AMPH on task behavior were assessed by administering rats injections of vehicle (saline; 1 ml/kg, i.p.) or AMPH (0.1, 0.3, 0.6, 1.0, and 2.0 mg/kg, i.p.) 5 min before they were placed in the operant

conditioning chamber for a test session. Injections were given over eight consecutive daily sessions in the order of VDDVDDVD (V = vehicle; D = drug). The order of drug doses was chosen based on a Latin square design, with a particular order assigned to each rat randomly. The exception to this was the 2.0 mg/kg dose of AMPH, which was administered during the last session because pilot studies suggested this dose produced significant motor impairments that potentially could influence task performance in subsequent sessions.

Open-field behavior retest. After the final session in the delay-discounting task, rats were given access to food *ad libitum.* After one week, all rats were re-tested in the open-field with a challenge injection of 3.0 mg/kg AMPH. The same procedures used during the initial open-field behavioral tests were followed, including the time of day experiments were performed.

Drugs. *d*-Amphetamine sulfate was obtained from Sigma-Aldrich (St. Louis, MO). It was dissolved in sterile saline (0.9% NaCl), and dosages were calculated based on the weight of the salt. All injections were given at a volume of 1 ml/kg.

Data Analyses. Data were imported into a relational database (SQL Server 2005 Developer, Microsoft; Seattle, WA) and analyzed using SAS 9.1 (SAS Institute, Inc.; Cary, NC) or SigmaStat 3.5 (Systat Software, Inc.; San Jose, CA). All graphs show group means ± SEM. The effects of saline or AMPH on open-field behavior were analyzed using two-way, mixed factor ANOVA, with group as the between-subjects factor and treatment day as the repeated measure. Because 3.0 mg/kg AMPH tends to produce stereotyped patterns of behavior (i.e., repetitive head movements or sniffing), we also used a semi-quantitative scoring method (adapted from Gulley et al. 2004) to

describe observed behavior. Raters blind to treatment conditions scored 30 sec segments of video taken every 5 min of the 60 min post-injection period. For each segment, the duration (0-30 sec) and intensity (1 – mild; 2 – moderate; 3 – intense) of focused stereotypy were recorded and these scores were then multiplied to give a single value (ranging from 0-90). These data were analyzed using three-way, mixed factor ANOVA with time and treatment day as within-subjects factors and group as the between-subjects factor. Follow-up analyses of significant interactions were performed with one- or two-way ANOVA and Holm-Sidak post-hoc tests.

For analysis of delay-discounting behavior, several measures were used. Choice behavior was represented as mean choice of the large reinforcer for free-choice trials only. The progression of task performance across sessions was assessed by obtaining these data during early acquisition (sessions 1-3), training midpoint (18-20), and stable baseline (sessions 41, 44, and 47). The stable baseline period encompassed the three sessions during which rats were given saline injections before they began the task. Data were analyzed with two-way repeated-measures ANOVA (treatment condition x delay). Data from tests of the acute effects of AMPH on task performance were analyzed using multifactorial ANOVA with treatment condition as a between-subjects factor and dose and delay as within-subjects factors. When appropriate, Holm-Sidak post-hoc tests were performed. Missing values were excluded from analysis. In cases where the assumption of sphericity was violated in repeated measures ANOVA, a multivariate analysis was used instead. In all tests, p values less than 0.05 were considered significant.

Results

Locomotor behavior

As expected, 3.0 mg/kg AMPH had robust effects on locomotor activity (Fig. 2.1). We observed significant main effects of group [$F(1,936) = 174$, $p<.001$], treatment day [$F(2,936) = 61.5$, $p<.001$], and time [$F(11,936) = 2.58$, $p<.01$] , along with a group x treatment day interaction [$F(2,936) = 255$, $p<.001$] and a three-way interaction [$F(22,936) = 6.01$, $p<.001$]. In the AMPH-pretreated group, the first drug exposure produced a significant increase in ambulation, compared to rats given saline (Day 2, Fig. 2.1A). After repeated AMPH treatment (10 injections; Day 20), however, ambulatory distance was significantly reduced compared to their first AMPH treatment day. This reduction, which was first apparent at 20 min following injection and became statistically significant at 45 min post injection, was due to the onset of focused stereotypy. As shown in Fig. 2.1B, the first exposure to AMPH led to increases in stereotypy that became maximal at ~50 min following injection. After repeated exposure, rats in the AMPH pretreatment group exhibited maximal stereotypy by 30-min post injection. Mixed-factor ANOVA of these data revealed significant main effects for group [$F(1,924) = 1420$, $p<.001$], treatment day [$F(2,924) = 217$, $p<.001$], and time [$F(11,924) = 44.8$, $p<.001$], with a group x treatment day interaction [$F(2,924) = 33.2$, $p<.001$], and a three-way interaction [$F(22,924) = 2.06$, $p<.001$].

One week after they finished testing in the delay-discounting task (see below), rats were re-tested in the open-field arena. During these tests, which occurred ~ 3 months after their last open-field test session (i.e., treatment day 20), rats in both groups were injected with 3.0 mg/kg AMPH in the open-field arena. Rats in the saline

pretreated group exhibited significant increases in both ambulation and stereotypy compared to their last saline test session (Fig. 2.1A and B). These effects were not significantly different from those observed in AMPH pretreated rats following their first AMPH injection, but it was different from drug-induced behavior in the AMPH pretreated group at re-test. In the AMPH-pretreated rats, there was no significant difference in ambulation at re-test compared to treatment day 20. Stereotypy was actually enhanced after the ~ 3 month time lapse between injections of this AMPH dose, with scores reaching maximal values earlier in the 60 min post-injection time period (Fig. 2.1B).

Delay-discounting behavior

As shown in Fig. 2.2, prior exposure to AMPH did not alter delay-discounting behavior. There were no significant differences between saline and AMPH treatment groups during the early phase of task acquisition (sessions 1-3), at the midpoint of training (sessions 18-20), and during baseline (sessions 41, 44, and 47). Rats in both groups did exhibit a robust sensitivity to delay by reducing their responding on the large reward lever as the delay increased to a maximum of 60 s. The main effect of delay was significant at all three training stages [acquisition: $F(4,24) = 4.33$, $p<.01$; midpoint: $F(4,24) = 22.0$, $p<.001$; and baseline: $F(4,24) = 73.5$, $p<.001$]. As shown in Fig. 2.2A, the sensitivity to delay was apparent at the earliest stages of task acquisition, although it was not as pronounced during the 0-20 s delays compared to later sessions (Fig 2.2B and C). The number of trial omissions was relatively low, with rats in saline and AMPH pre-treatment groups omitting a total of 0.376 ± 0.114 and 0.196 ± 0.11 trials, respectively, across all delays during the midpoint sessions. This small group

difference was not statistically significant, and neither were the small group differences in trial omissions at acquisition [saline: 1.18 ± 0.39; AMPH: 0.97 ± 0.38] and baseline [saline: 0.31 ± 0.09; AMPH: 0.16 ± 0.09].

When AMPH was administered 5 min before the start of the task, it induced a dose-dependent change in choice behavior [dose: $F(5,810) = 20.7$, $p<.001$)] that varied as a function of delay [dose x delay: $F(4,810) = 117$, $p<.001$], but was not significantly different in saline and AMPH pretreated rats. Subsequent analysis of AMPH effects in the different treatment groups with two-way ANOVA and post-hoc analysis revealed that the 2.0 mg/kg AMPH significantly decreased choice of the large reward at the 0 and 10 s delays for saline pretreated rats and the 0, 10 and 20 s delays for those pretreated with AMPH (Fig. 2.3A-B). The number of trial omissions (Fig. 2.3C) and latencies to nosepoke or lever press (Fig. 2.4), in contrast, were increased over a wider range of AMPH doses and there were differential effects in the pretreatment groups. For omissions, we observed significant main effects of group [$F(1,810) = 4.19$, $p<.05$], dose [$F(5,810) = 155$, $p<.001$], and a group x dose interaction [$F(5,810) = 50.3$, $p<.001$]. Pairwise comparisons revealed that the 1.0 and 2.0 mg/kg doses were significantly different from vehicle, and the 2.0 mg/kg dose produced the most robust effect. The effects of the highest dose were most pronounced in the AMPH-pretreated group.

The latency to nosepoke for presentation of levers, as well as to respond on a lever, was increased by AMPH to a similar extent in both groups (Fig. 2.4A). There were significant main effects of dose for both measures [nosepoke latency: $F(5,22) = 3.68$, $p<.01$; lever press latency: $F(5,16) = 3.53$, $p < .05$], with the 2 mg/kg dose increasing latency to the greatest extent compared to vehicle injections. The number of

nosepoke responses that rats made during periods when food pellets were not available—during ITIs and during delays following responses on the large reward lever—were decreased by all doses of AMPH (Fig. 2.4B and C). For nosepoke responses during ITIs and delays, only the main effects of dose were significant [ITIs: $F(5,23) = 8.64$, $p<.001$; delays: $F(5,23) = 4.37$, $p<.01$]. More specifically, all doses of AMPH reduced the number of nosepoke responses compared to vehicle sessions. This effect tended to be greater in AMPH-pretreated rats, but the main effect of group and the group x dose interaction were not significant. Also, the saline-pretreated group made more nosepoke responses during ITIs than the AMPH-pretreated group after vehicle and the highest tested doses of AMPH (Fig. 2.4B), although this effect was not statistically significant.

Discussion

Repeated exposure to 3.0 mg/kg AMPH produced robust changes in AMPH-induced locomotor activity in an open-field arena that was evident following 10 injections in 20 days. This treatment did not, however, alter baseline choice behavior in a delay discounting task. There were subtle effects on task performance when AMPH was given prior to the sessions, but these were likely due to motor disruptions produced by the higher doses and may be a reflection of the higher sensitivity to AMPH's locomotor effects that were observed in the open-field for this group. When rats in the AMPH pretreatment were given an AMPH challenge in the open-field after the conclusion of their final delay-discounting session—a procedure that occurred ~3 months after their last injection in the open-field—the sensitization to AMPH's stereotypy-inducing effects

was still evident and slightly enhanced. Rats in the saline pretreatment group exhibited AMPH-induced behavior that was not significantly different from that observed in the AMPH pretreatment group following their first drug injection. Taken together, these findings suggest that the long-lasting locomotor effects of repeated, intermittent AMPH exposure can be dissociated from effects on delay-discounting behavior. Furthermore, our results suggest that the neuroadaptations that resulted from repeated AMPH exposure, which are known to be associated with sensitization (Robinson and Berridge 2000), do not cause enduring deficits in the aspects of cognition that the delay-discounting task requires. It is not clear from the current study if the observed dissociation is specific to the dosing procedure used, but it is notable that previous studies using similar AMPH treatment schedules have reported a reduction in subsequent structural plasticity and forms of new learning (Kolb et al. 2003; Briand et al. 2005), as well as impairments in object recognition memory (Bisagno et al. 2003; Belcher et al. 2006).

With respect to the long-lasting sensitization that was observed in AMPH pretreated rats, it should be noted that these rats received an additional cumulative dose of 4.0 mg/kg AMPH across the 8 days of drug and vehicle testing in the delay-discounting task. The saline pre-treated group also had this same level of exposure, however, and their response to AMPH challenge was no different from that seen in AMPH pretreated rats after their first drug exposure. Thus, this additional exposure to AMPH is unlikely to account for the persistence of sensitization. Similar long-lasting changes in AMPH-induced behavior have been observed previously (Segal and Mandell 1974; Klawans and Margolin 1975; Kolta et al. 1985; Robinson and Becker 1986).

To our knowledge, no published studies have described the effects of chronic AMPH exposure on delay-discounting behavior in an animal model, but several have examined other psychostimulants (Richards et al. 1999; Paine et al. 2003; Roesch et al. 2007; Simon et al. 2007; Winstanley et al. 2007). While most of these reports suggested that repeated psychostimulant exposure increases impulsive decisions, the most extensive study to date (Winstanley et al. 2007) reported no baseline impairments in impulsive choice in rats exposed repeatedly to cocaine (15 mg/kg, i.p., for 21 days). Unfortunately, they did not report locomotor behavior, or more specifically, if behavioral sensitization was observed. As for the remaining studies, there are key factors that differ between each of them and ours. In the studies by Richards et al. (1999) and Paine et al. (2003), drug treatment began after training in the task was completed and injections were given following daily delay-discounting sessions. Interestingly, Paine et al. (2003) reported that increased impulsive choice in rats was only observed in association with behavioral sensitization (i.e., on day 7 of cocaine treatment; 15 mg/kg, 3 injections/day for 14 days), and choice behavior returned to baseline levels on subsequent days even though sensitization persisted or was enhanced. In a study by Simon et al. (2007) that utilized chronic treatment with a high dose of cocaine (30 mg/kg daily for 14 days), cue lights did not remain illuminated during the delay periods that occurred following responses on the large reward lever. The absence of cues has previously been shown to decrease rat's choices of the large reward lever (Cardinal et al. 2000), which is what the authors reported.

Simon et al. (2007) also observed a reduction in the number of nosepoke responses into the food trough during the delay periods prior to reward delivery.

Interestingly, we did not observe this difference during vehicle sessions. At all tested doses of AMPH, however, we did find this effect. This is likely a separate construct from impulsive choice (Evenden 1999) and has been referred to as impulsive action. A few studies have addressed the influence of chronic AMPH exposure on impulsive action, and a task commonly used to test this characteristic is differential reinforcement of low rates of responding. Using this task, increased impulsive action was observed in AMPH-treated rats immediately following treatment (5.0 mg/kg for 9 days), but this only persisted for 9 days after the cessation of drug exposure (Peterson et al. 2003). Once again, these findings suggest that impulsive behavior is only transiently altered by repeated AMPH exposure.

In the present study, injections of 0.1-1.0 mg/kg AMPH had subtle effects on choice behavior in both pre-treatment groups. The 2.0 mg/kg dose increased the number of omissions and latencies to both nosepoke and lever press, with the AMPH-pretreated group exhibiting significantly more omissions than the saline-pretreated group. There are several reports indicating that acute AMPH treatment either dose-dependently reduces (Cardinal et al. 2000; deWit et al. 2002; Winstanley et al. 2003; vanGaalen et al. 2006), increases (Evenden and Ryan 1996; Cardinal et al. 2000), or has little to no effect (Uslaner and Robinson 2006) on impulsive choice in a delay-discounting paradigm. It is unclear why the results of these various studies are somewhat equivocal, but differences in baseline choice behavior have been shown to influence the effects of AMPH on delay discounting. For example, Winstanley et al. (2003) have suggested that AMPH decreases impulsive choice in rats that tend to have higher levels of impulsive choice before drug treatment. A recent study (Barbelivien et

al. 2008), which systematically examined the interaction between baseline choice behavior and AMPH's effects on delay discounting, revealed that AMPH significantly decreased impulsive choice in rats with a "medium" basal level of impulsive choice and had no significant effect in those with either low or high baseline levels. In the present study, our sample was not sufficiently large to appropriately analyze sub-populations of rats with diverse choice behavior at baseline, but we did note a trend for AMPH to decrease impulsive choice most in rats with high to medium baseline levels, and an opposite effect in animals with low baseline levels of impulsive choice.

While current theories of drug addiction suggest that heightened impulsivity is a common characteristic of individuals who abuse drugs (Jentsch and Taylor 1999; Lyvers 2000; Goldstein and Volkow 2002; Bechara 2005), it is not clear whether this is a major contributing factor to the development of addiction or if it is caused by repeated drug exposure. Our study in rats suggests that increased impulsive choice is not an inevitable consequence of repeated, intermittent exposure to AMPH. Instead, it supports the notion that, at least in rats, a high degree of impulsivity is more likely a pre-existing trait that is potentially amplified by exposure to drugs (Bechara 2005; Dalley et al. 2007). Similarly, evidence from clinical studies (Ersche et al. 2008) suggests that repeated drug exposure does not lead to generalized effects: chronic cocaine use was associated with perseverative responding, whereas chronic amphetamine use was not. Taken together, these studies and our results underscore the importance of avoiding generalization between drugs of abuse and ensuing changes in behavior and the brain. Furthermore, our study suggests that the neuroadaptations that are known to be associated with the long-lasting expression of behavioral sensitization (Robinson and

Berridge 2000) may not have wide-ranging consequences for other behaviors that are considered to be associated with, or characteristic of, addiction. Rather, there may be a unique or potentially overlapping set of drug-induced neuroadaptations that lead to heightened impulsivity.

CHAPTER 3: DISPARATE COCAINE-INDUCED LOCOMOTION AS A PREDICTOR OF CHOICE BEHAVIOR IN RATS TRAINED IN A DELAY-DISCOUNTING TASK[2]

Abstract

Heightened impulsivity and differential sensitivity to a drug's behavioral effects are traits that, individually, have been associated with chronic drug use and dependence. Here, we used an animal model to test whether individual differences in cocaine-induced activity are predictive of impulsive choice behavior. Adult, male Sprague-Dawley rats were given cocaine (10 mg/kg, i.p.) and classified into low or high cocaine responders (LCRs and HCRs, respectively) based on their locomotor response in an open-field arena. Rats were then trained in a delay-discounting task that offers a choice between immediately delivered, but smaller reinforcements, or larger reinforcements that are delivered after a delay. We also examined the effects of amphetamine (AMPH; 0.3-1.0 mg/kg) and the 5-HT_{1A} agonist 8-OH-DPAT (0.3-1.0 mg/kg) on delay discounting. Lastly, all rats were retested in the open-field to determine if phenotypes were stable. We observed baseline differences in choice behavior between the groups, with HCRs behaving more impulsively (i.e., choosing the small reinforcement) compared to LCRs. AMPH decreased choice of the large reinforcement in LCRs, but did not alter choice in HCRs. Impulsive choice was increased in both phenotypes following 8-OH-DPAT, with LCRs exhibiting changes across a wider range of delays. When cocaine-induced open-field behavior was retested, responses in LCRs

[2] This chapter appeared in its entirety in the journal Drug and Alcohol Dependence as Stanis JJ, Burns RM, Sherrill LK, Gulley JM (2008) Disparate cocaine-induced locomotion as a predictor of choice behavior in rats trained in a delay-discounting task. 98(1-2):54-62. This article is reprinted with the permission of the publisher and is available from http://www.sciencedirect.com and using DOI: 10.1016/j.drugalcdep.2008.04.009

were similar whereas HCRs showed evidence of tolerance. Our results suggest that differential sensitivity to cocaine-induced locomotion is predictive of impulsivity and the potential neurobiological differences in LCRs and HCRs may provide insight into mechanisms contributing to vulnerability for chronic drug use and/or dependence.

Introduction

Impulsivity is a complex, multifaceted trait that is broadly defined by a lack of behavioral inhibition, which includes premature and poorly controlled actions, and impulsive choice, where decisions are poorly conceived and sensitive to delayed rewards (see Evenden, 1999; Winstanley et al., 2006a for more detailed accounts). Individuals who abuse drugs tend to exhibit heightened levels of these traits, particularly with regards to impulsive decision-making (Bornovalova et al., 2005; Coffey et al., 2003; Heil et al., 2006; Kirby and Petry, 2004; Lejuez et al., 2007; Moeller et al., 2002; Verdejo-Garcia et al., 2007). It has been suggested that a high level of impulsivity develops as a result of repeated exposure to abused drugs, and this in turn facilitates the development and/or maintenance of addiction (Jentsch and Taylor, 1999). Evidence supporting this hypothesis comes in part from studies using animal models, where repeated exposure to cocaine in rats leads to an increase in impulsive choice behavior relative to that observed in saline-treated controls (Paine et al., 2003; Roesch et al., 2007; Simon et al., 2007), and from clinical studies, where individuals who have been chronically exposed to cocaine show increases in impulsive choice relative to non-drug users or drug abstainers (for review, see Bickel and Marsch, 2001 and Reynolds, 2006).

An alternative and not necessarily mutually exclusive hypothesis is that a high level of impulsivity is a pre-existing trait whose multidimensional components overlap with other traits or behaviors that also confer enhanced vulnerability to chronic drug use and/or dependence (Bechara, 2005; Dalley et al., 2007a, b; Kreek et al., 2005). An example of one such candidate trait is differential sensitivity to a drug's behavioral effects. In humans, for example, differences in initial sensitivity to cocaine are predictive of long-term use and dependence (Davidson et al., 1993; Haertzen et al., 1983; Schafer and Brown, 1991), and a recent study has indicated that individuals who reported a high degree of "liking" or "wanting" on their initial use of cocaine had a significantly increased risk of cocaine abuse (Lambert et al., 2006). In rodents, a reliable indicator of initial sensitivity to psychostimulants is drug-induced locomotor activity in an open-field arena. Cocaine, in particular (Briegleb et al., 2004), has variable stimulant effects on behavior, such that outbred rats can readily be classified as low or high cocaine responders (LCRs or HCRs, respectively) based on their response to a single treatment with cocaine (Sabeti et al., 2002; Gulley et al., 2003; Gulley, 2007). Interestingly, these individual differences are related to differences in the function of dopamine transporters (DATs) in the dorsal striatum and nucleus accumbens (Sabeti et al., 2002, 2003), and not to pharmacokinetic factors (Gulley et al., 2003). Subsequent studies have shown that the LCR/HCR phenotype can be used to predict behavior in a food-reinforced operant task (Gulley, 2007), as well as conditioned place preference for cocaine (Allen et al., 2007).

In the current study, we used rats to examine if differential sensitivity to the locomotor activating effects of cocaine were predictive of one component of impulsivity,

impulsive choice behavior. Rats were first characterized as LCRs and HCRs based on their response to 10 mg/kg cocaine in an open-field arena. They were then trained in a delay-discounting task that offered a choice between immediately delivered, but smaller reinforcements, or larger reinforcements that were delivered after a delay of up to 60 s. After 34 daily training sessions, we performed a series of drug challenges in order to determine if impulsive choice could be altered in a differential manner between LCRs and HCRs. First, rats were given amphetamine (AMPH; 0.3-1.0 mg/kg) or saline prior to the start of daily delay-discounting sessions. AMPH has been shown previously to increase or decrease delay-discounting behavior (Cardinal et al., 2000; Evenden and Ryan, 1996, 1999; van Gaalen et al., 2006), and it has been suggested that an individual's baseline level of impulsive choice behavior influences this response (Barbelivien et al., 2008; Winstanley et al., 2003). Rats were then challenged with the 5-HT_{1A} agonist 8-OH-DPAT (0.3-1.0 mg/kg) or saline prior to their daily session. 8-OH-DPAT has been shown to increase impulsive choice (Winstanley et al., 2005; but see Evenden and Ryan, 1999; Poulos et al., 1996) and it is not clear if this effect depends on the baseline level of impulsive choice. Lastly, all rats were re-tested in the open-field for their response to cocaine in order to assess if the phenotypic differences in cocaine response that were established initially remained stable following the ~3 months time period that elapsed during the course of the study.

Methods

Animals. Male Sprague-Dawley rats (n = 16), bred in our animal facility from stock rats obtained from Harlan (Indianapolis, IN), were housed individually starting at

~2 months of age and were 3-3.5 months old (300-490 g) at the start of experiments. They were maintained on a 12:12 h light: dark cycle (lights on at 0800) with experimental sessions conducted between 0900 and 1800 h. Rats were handled five times for 15 min intervals prior to being used in experiments. With the exception of periods when rats were undergoing operant training and testing, food was available *ad libitum*. Water was always available *ad libitum*. All experimental procedures were approved by the IACUC at the University of Illinois, Urbana-Champaign and were consistent with the *Principles of Laboratory Animal Care* (NIH Publication no. 85-23).

Apparatus. Locomotor activity was measured in an open-field chamber that consisted of a transparent, Plexiglas box (40.6 x 40.6 x 40.6 cm) surrounded by photobeams (Coulbourn Instruments; Allentown, PA). Each apparatus was connected to a computer operating software (TruScan, v. 2.01; Coulbourn Instruments) that recorded all horizontal and vertical beam breaks (100 ms sampling rate). The horizontal beam breaks, or coordinate changes, were converted into distance traveled (cm). The chambers were individually contained inside sound-attenuating cubicles (76 x 80 x 63 cm). Each cubicle contained a speaker (76 mm dia.) fixed to one wall, two ceiling-mounted white lights (4 W) for dim illumination, and a ceiling-mounted camera between the two lights. White nose (70 dB) was played continuously through the speakers when rats were in the testing room.

Operant behavior was monitored in standard operant chambers (Coulbourn Instruments). One wall of the chamber contained two retractable levers that were positioned on either side of a centrally located food trough. Infrared detectors were used to monitor head entries into the food trough. White cue lights were located above

each lever. A white houselight was located near the top of the chamber on the opposite wall.

Initial behavioral characterization: Locomotor activity in the open-field. After a 30-min acclimation period in the testing room, rats were placed in the open-field chambers for 90 min. They were then removed from the chamber, injected (i.p.) with (-) cocaine HCl (10 mg/kg) and placed back into the chamber for an additional 60 min. This dose was chosen based on previous studies (Sabeti et al, 2002; Gulley et al, 2003) showing that it is optimal for inducing the widest range of behavioral responses in male Sprague-Dawley rats. After the testing session, rats were returned to the colony room and their access to food was restricted so that they were reduced to 85% of their free-feeding weight over the course of several days. Rats were maintained at 85-90% of free-feeding weight for the duration of operant training and testing.

Delay-discounting behavior. Seven days after the open-field test, rats were trained in overnight sessions (2100 to 0900 hours) to respond on either of two available levers for a food pellet (45-mg; Bio-Serv F0021 or F0042) on a fixed ratio schedule (FR1) of reinforcement. After they displayed approximately equal responding on both levers, rats were moved to the next training phase (1-h sessions, between 0900 and 1700 h). Trials began with levers retracted, and the food trough illuminated by a cue light. A nosepoke into the trough resulted in the extension of one randomly selected lever, with a subsequent lever press response reinforced by delivery of a food pellet. After 3-4 days of training at this stage, rats began the final stage of training.

Training in the delay-discounting task was done in daily, 100 min sessions that consisted of five blocks of 12 trials. Each trial lasted 100 s and began with the

illumination of the house light and cue light located in the food trough. Rats were required to nosepoke into the trough within 10 s, whereupon a single lever would be presented randomly and a response was reinforced with food pellet delivery. The amount of food delivered was pre-assigned to a lever, such that responses on one (e.g., left side lever) resulted in a larger, delayed reward of four pellets and responses on the other (e.g., right side lever) resulted in a small, immediate reward of one pellet. Assignment of reward magnitude to levers was counterbalanced across groups, but remained consistent for each rat. After a lever response, the house light turned off, the levers retracted, and the cue light above the lever was illuminated until food was delivered. If the rat either failed to nosepoke within 10 s or respond on the presented lever, the trial was recorded as an omission. On omission trials, the levers remained retracted and the chamber was returned to an intertrial interval (ITI) state until the beginning of the next trial. After completing or omitting the first two forced-choice trials (which served as exemplars for each block), 10 free-choice trials were presented where both levers were extended. Delays for the delivery of large rewards increased with each block of trials (0, 10, 20, 40, and 60 seconds, respectively). Each animal participated in one session per day, for a minimum of 34 training sessions.

Following the last training session, the effects of AMPH or 8-OH-DPAT on task behavior were assessed in two testing blocks. For the first testing block, rats were given injections of vehicle (saline; 1 ml/kg, i.p.) or AMPH (0.3, 0.6, 1.0 mg/kg, i.p.) 5 min before they were placed in the operant chamber for a test session. Saline or drug injections were given in an alternating fashion over six consecutive daily sessions (i.e., SDSDSD, where S = saline and D = drug). The order of drug doses was chosen based

on a Latin square design, with a particular order assigned to each rat randomly. Rats were given a five day break after the last AMPH test session. Subsequently, they were allowed a session without any injections, followed on the next day by the start of the second testing block. For these tests, saline or 8-OH-DPAT (0.3, 0.6. and 1.0 mg/kg, i.p.) was given using the same procedure as was used in the first testing block.

Open-field behavior retest. After their final session in the delay-discounting task, rats were given access to food *ad libitum.* One week later, they were re-tested in the open-field with 10 mg/kg cocaine (i.p.). The same procedures used during the initial open-field behavioral characterization test were followed, including the time of day experiments were performed.

Drugs. The (-) cocaine HCl used in this study was obtained from NIDA (RTI International; Research Triangle Park, NC). *d*-Amphetamine sulfate and 8-OH-DPAT were obtained from Sigma-Aldrich (St. Louis, MO). Drugs were dissolved in sterile saline (0.9% NaCl), with dosages calculated based on the weight of the salt. All injections were given at a volume of 1 ml/kg.

Data Analyses. All analyses were conducted using SAS 9.1 (SAS Institute, Inc.; Cary, NC), SigmaStat 3.5 (Systat Software, Inc.; San Jose, CA), and SQL Server 2005 Developer (Microsoft; Seattle, WA). Cocaine-induced locomotor activity in the open-field was assessed by determining the cumulative activity during the first 30-min following injection. As in previous studies (Gulley et al., 2003; Gulley, 2007), rats with activity scores in the lower half of the population distribution were designated LCRs and those in the upper half were designated HCRs. The statistical significance of group differences in cocaine-induced activity was determined using two-way, mixed factor

ANOVA, with group as the between-subjects factor and test session as the repeated measure. The locomotor response to a novel environment was also assessed by determining the cumulative activity during the first 30-min following placement into the open-field arena; group differences were analyzed with a one-way ANOVA.

Several measures were used to analyze delay-discounting behavior. Choice behavior was represented as mean choice of the large reward for free-choice trials only. The progression of task performance across sessions was assessed by obtaining these data during early acquisition (sessions 1-3), training midpoint (15-17), and stable baseline (sessions 35, 37, and 39). The stable baseline period encompassed the three sessions during which rats were given saline injections before they began the task. Data from each of these training endpoints were analyzed with two-way repeated-measures ANOVA (group x delay, as well as session x delay for sessions 32-34). In order for behavior to be considered stable, the session factor had to be non-significant, while the delay factor had to be significant. Data from tests of the acute effects of AMPH or 8-OH-DPAT on task performance were analyzed using multifactorial ANOVA with group as a between-subjects factor and dose and delay as within-subjects factors. Further ANOVAs were performed (with session and delay as within-subject factors) to test for stability in behavior between vehicle injections. When appropriate, Holm-Sidak post-hoc tests were performed. In all tests, *p* values less than 0.05 were considered significant.

Results

Open-field behavior

The first exposure to 10 mg/kg cocaine resulted in disparate locomotor activity that could be described by separating rats into LCRs and HCRs. As shown in Fig. 3.1, LCRs exhibited, on average, 61% less cocaine-induced activity compared to that observed in HCRs. This group difference in behavior was statistically significant [main effect of group: $F(1,14) = 27.0$, $p < 0.001$]. Novelty-induced activity was also lower in LCRs (10,206 ± 895 cm) compared to HCRs (12,822 ± 854 cm), but this difference was not statistically significant. Following the completion of training and testing in the delay-discounting task (see below), rats were retested in the open-field arena for their response to 10 mg/kg cocaine. During the ~3 month period that elapsed between the first and second tests, these rats were never exposed to the open-field chambers or the testing room in which the chambers were housed. As shown in Fig. 3.1, we found that cocaine-induced activity in LCRs was similar in both tests. HCRs, in contrast, exhibited less activation on retest compared to their initial characterization. These effects were reflected in a significant interaction between group and test session [$F(1,14) = 6.47$, $p < 0.05$]. Cocaine-induced activity in LCRs and HCRs was not significantly different on retest.

Delay-discounting behavior

As shown in Fig. 3.2, there were differences in delay-discounting behavior between LCRs and HCRs. We observed significant main effects of group [$F(1,210) = 29.4$, $p < 0.001$], training period [$F(2,210) = 11.3$, $p < 0.001$], and delay [$F(4,210) = 29.5$, $p < 0.001$]. More specifically, group differences in delay-discounting were significant during the midpoint of training [sessions 15-17; group x delay interaction: $F(4,56) =$

37.6, $p < 0.05$] and at stable baseline [sessions 35, 37, and 39; group x delay interaction: $F(4,56) = 30.7$, $p < 0.01$], but not during the early phase of task acquisition (Fig. 3.2A-C). As expected, rats in both groups exhibited sensitivity to delay by reducing their responding on the large reward lever as their number of days in training increased. As shown in Fig. 3.2A, the sensitivity to delay was emerging in the earliest stages of task acquisition, primarily at the 40- and 60-s delays. As training progressed, rats in both groups chose the large reward almost exclusively when there was no delay, and progressively less often as delay increased. Furthermore, discounting was less steep in LCRs compared to HCRs. This was most apparent at the midpoint of training, where the groups differed at the 10, 20, and 40 s delays (Fig. 3.2B). At stable baseline, LCRs continued to choose the large reward more frequently than HCRs across these same delays, although the difference at 20 s was the only one that was statistically significant (Fig. 3.2C). The number of trial omissions was relatively low for both groups, with LCRs and HCRs omitting a total of 0.36 ± 0.27 and 0.33 ± 0.27 trials, respectively, across all delays during the midpoint sessions. The small group difference was not statistically significant, and neither were those at acquisition (LCRs: 1.81 ± 0.74 trials; HCRs: 0.65 ± 0.74 trials) or baseline (LCRs: 0.08 ± 0.06 trials; HCRs: 0.09 ± 0.06 trials).

Effects of AMPH on delay-discounting

When AMPH was administered 5 min before the start of the task, it significantly altered choice behavior (Fig. 3.3). Overall ANOVA revealed significant main effects of group [$F(1,280) = 18.3$, $p < 0.001$] and delay [$F(4,280) = 50.8$, $p < 0.001$], as well as a group x delay interaction [$F(4,280) = 3.20$, $p < 0.01$]. Subsequent analysis of within-

group AMPH effects using a two-way ANOVA and post-hoc tests revealed dose-dependent changes in LCRs [dose: $F(3,84) = 3.28$, $p < 0.05$], with a trend for a dose x delay interaction [$F(12,84 = 1.81$, $p = 0.06$]. As shown in Fig. 3.3A for LCRs, 1.0 mg/kg AMPH decreased choice of the large reward compared to that observed following vehicle injection. The most robust effect was at the 20-s delay. There were no significant effects of any of the tested AMPH doses on choice behavior in HCRs. For number of trial omissions, overall ANOVA revealed a significant main effect of dose [$F(3,280) = 10.6$, $p < 0.001$] and a delay x dose interaction [$F(12,280) = 3.05$, $p < 0.001$]. Subsequent analysis revealed that the 1.0 mg/kg dose of AMPH was significantly different from vehicle, but only in HCRs and only at the 20-s delay (Fig. 3.3C).

The latency to nosepoke for the presentation of levers, as well as latency to respond on a lever, was altered to a modest extent by AMPH. For these analyses, data were collapsed across delay because latencies remained relatively stable across the session. We observed significant main effects of dose for both measures [nosepoke latency: $F(3,42) = 6.13$, $p < 0.001$; lever press latency: $F(3,42) = 2.94$, $p < 0.05$]. As shown in Table 3.1, the 0.3 mg/kg dose significantly decreased response latencies in LCRs. The number of nosepoke responses that rats made during periods when food pellets were not available—during ITIs and during delays following responses on the large reward lever—was not significantly different between LCRs and HCRs following vehicle injection or after AMPH treatment (data not shown).

Effects of 8-OH-DPAT on delay-discounting

When 8-OH-DPAT was administered 5 min before the start of the task, it significantly reduced choice of the large reward, with a relatively greater effect in LCRs (Fig. 3.4A). An overall ANOVA revealed significant main effects of group [$F(1,280) = 34.2$, $p < 0.001$], dose [$F(3,280) = 12.3$, $p < 0.001$], and delay [$F(4,280) = 37.0$, $p < 0.001$]. Subsequent analysis of 8-OH-DPAT within groups revealed that the two highest doses significantly decreased choice of the large reward in LCRs, and in particular, at the 0-, 10-, and 20-s delays. The effect of 1.0 mg/kg 8-OH-DPAT was also significant at the 40-s delay. In HCRs, all three doses reduced responding on the large reward lever, but this effect was only significant at the 0-s delay (Fig. 3.4B). The changes in choice behavior produced by 8-OH-DPAT were associated with increases in trial omissions, which were evident in both groups at the 0.6 and 1.0 mg/kg doses (Fig. 3.4C). Statistical analysis revealed a trend for a main effect of dose in LCRs [$F(3,84) = 2.57$, $p = 0.08$] and a significant main effect of dose [$F(3,84) = 4.99$, $p < 0.01$] and a delay x dose interaction [$F(12,84) = 3.93$, $p < 0.001$] in HCRs.

The latency to nosepoke for the presentation of levers, as well as to respond on a lever, was also altered by 8-OH-DPAT. Similar to the analysis of AMPH effects, these data were collapsed across delay because latencies remained stable across the session. We found significant main effects of dose for both measures [nosepoke latency: $F(3,42) = 8.14$, $p < 0.001$; lever press latency: $F(3,37) = 6.31$, $p < 0.05$], with the 0.6 and 1.0 mg/kg doses having significant effects compared to vehicle injections (Table 3.1). There was no significant effect of 8-OH-DPAT on the total number of nosepoke responses that rats made during periods when food pellets were not available.

Discussion

A primary goal of this study was to investigate a potential overlapping relationship between individual differences in the initial sensitivity to cocaine-induced behavior and impulsive choice. Using a delay-discounting operant procedure, we observed baseline differences in impulsive choice behavior in rats with disparate locomotor responses to 10 mg/kg cocaine in an open-field arena. Specifically, HCRs showed a greater sensitivity to delay, and thus behaved more impulsively, compared to LCRs. When AMPH was administered prior to delay-discounting sessions, we found that the highest dose tested (1.0 mg/kg) increased impulsive choice in LCRs without influencing the number of trial omissions. None of the tested doses had a statistically significant effect on impulsive choice in HCRs, although the highest dose significantly increased trial omissions. When rats were tested for the effects of 8-OH-DPAT, which occurred one week after the last AMPH test session, we found significant increases in impulsive choice in both phenotypes, although HCRs were only affected at the 0-s delay. Taken together, our results suggest that initial sensitivity to the locomotor effects of cocaine can be used to predict subsequent behavior in a delay-discounting task that examines trait behavior associated with drug use and dependence (Dalley et al., 2007a). Furthermore, they show that drugs previously shown to alter impulsive choice in rats (e.g., Cardinal et al., 2000; Evenden and Ryan, 1996, 1999; van Gaalen et al., 2006; Winstanley et al., 2005) have differential effects in LCRs compared to HCRs.

While ours is the first published study to assess the relationship between individual differences in cocaine-induced locomotion and impulsivity, several studies have investigated individual differences in response to novelty as a predictor of

impulsive behavior (Bardo et al., 2006; Dellu-Hagedorn, 2006; Stoffel and Cunningham, 2008). For example, Stoffel and Cunningham (2008) examined the relationship between locomotor activation induced by exposure to an inescapable novel environment and impulsive behavior in a differential reinforcement of low rates of responding (DRL) task. They found that novelty phenotype was predictive of the behavioral inhibition displayed, such that the high novelty responders (HRs) were more impulsive than low novelty responders (LRs). In addition, HRs and LRs were equally sensitive to cocaine-induced increases in impulsivity. Although it was not the focus of the present study, we analyzed the relationship between novelty responses and impulsivity by re-characterizing our rats as LRs or HRs based on their locomotor behavior during the first 30 min of the habituation period on their first open-field test. We found no significant differences in delay-discounting behavior in LRs and HRs (data not shown). Notably, the lack of overlap between the novelty and cocaine response phenotypes has been described previously in male Sprague-Dawley rats (Gulley et al., 2003; Gulley, 2007), and suggests the LCR/HCR classification is somewhat distinct from one based on novelty and may be mediated by different neural mechanisms. It is also noteworthy that different methods of assessing impulsive behavior in rats (e.g., delay-discounting compared to DRL tasks) are likely to be focusing on particular aspects of impulsivity, and the neural mechanisms of impulsive behavior are not likely to be identical (Evenden, 1999; Winstanley et al., 2006a).

AMPH had differential effects on impulsive choice in our tests, with increases observed in LCRs and no effect observed in HCRs. These results were somewhat surprising given previous reports that suggested AMPH, at the doses tested here,

decreases impulsivity (Barbelivien et al., 2008; Cardinal et al., 2000; deWit et al., 2002; Winstanley et al., 2003; van Gaalen et al., 2006). However, AMPH's effects on delay-discounting behavior are not unequivocal; it has been shown to increase (Evenden and Ryan, 1996; Cardinal et al., 2000) or have little to no effect on impulsive choice (Barbelivien et al., 2008; Uslaner and Robinson, 2006). One factor that has been suggested as a potential contributor to these inconsistent results is differences in the baseline level of delay-discounting that rats exhibit. In a previous study (Winstanley et al., 2003), AMPH was most effective at altering task behavior in rats that expressed a high baseline level of impulsivity. In our study, however, HCR rats showed the greatest sensitivity to delay at baseline and AMPH had no significant effect on their choice of the large reward across delays. These results are consistent with those from a recent study by Barbelivien et al. (2008). AMPH did increase the number of trial omissions at the highest dose, which is consistent with an enhanced sensitivity to AMPH-induced disruptions in motor behavior that might be expected in rats with high locomotor responses to cocaine. Interestingly, in that same study (Winstanley et al., 2003), the authors reported that AMPH's effects on impulsive choice were attenuated in a group of rats that were depleted of their forebrain 5-HT levels. Thus, impulsive choice might not only depend on baseline levels of impulsivity, but also on intact neurotransmitter systems. The results from the present study do offer some insight into the consequences of the neurobiological differences between LCRs and HCRs. Because the DAT is a primary pharmacological target of AMPH, and alterations in DA signaling are important for behavior in the delay-discounting task (Floresco et al., 2007; van Gaalen et al., 2006), the differential effects we observed for AMPH in LCRs compared

to HCRs are a likely reflection of known differences in DAT function between these phenotypes (Sabeti et al., 2003; Briegleb et al., 2004). Importantly, impulsive choice behavior may be influenced by an interaction between DAergic and 5-HTergic function (Winstanley et al., 2005, 2006b), and this could be relevant for differences in choice behavior between LCRs and HCRs.

Direct stimulation of the 5-HT_{1A} receptor through administration of the selective agonist 8-OH-DPAT reduced responding for the large reinforcement in both groups, with LCRs choosing it less frequently across a wide range of delays (10-40 s) at both the 0.6 and 1.0 mg/kg doses. The effect in HCRs was seen at all three tested doses, but only when there was no delay in reinforcement delivery. Increases in impulsive choice following 1.0 mg/kg 8-OH-DPAT have been reported previously (Winstanley et al., 2005), and this effect is partly related to the drug's activation of both postsynaptic 5-HT receptors and somatodendritic autoreceptors. The lack of effect at the 10-60 s delays in HCRs may be due to a floor effect as these rats were already demonstrating steep delay discounting curves (i.e., highly impulsive) at baseline. Additional studies will be necessary to determine if differences in 5-HT systems between LCRs and HCRs contribute to some of the differences in behavior in these phenotypes. Noteworthy in this regard are a number of recent studies highlighting the importance of 5-HT systems in cocaine-induced locomotor activity (Bubar and Cunningham, 2006; Carey et al., 2001, 2005; Liu and Cunningham, 2005), as well as timing processes (Ho et al., 2002) and sensitivity to delayed reinforcement (Mobini et al., 2000; Wogar et al., 1993). It will also be important to determine if the effects of 8-OH-DPAT on delay-discounting are

influenced by changes in other behavioral processes, such as altered motivation for feeding or disrupted motor processes.

After the conclusion of the final delay-discounting sessions – which was approximately three months after the first cocaine injection was given – rats were retested in the open-field with 10 mg/kg cocaine. The behavior of LCRs was not significantly different on retest compared to the first test, whereas HCRs exhibited a relative decrease in cocaine-induced locomotor activity. The stability of the LCR phenotype, and the apparent tolerance evident in HCRs, is consistent with a previous study where LCRs and HCRs were retested following a seven day period where they were given extensive habituation to the testing environment (Gulley et al., 2003). In the present study, it is possible that either the AMPH and/or 8-OH-DPAT treatment in the context of the delay-discounting task had an effect on subsequent locomotor behavior in the open-field retest. This is unlikely for several reasons. First, the retest was given a week after the last drug treatment to prevent any carry-over effects. Second, administration of these drugs at low to moderate doses tends to potentiate locomotor activity when given alone or prior to cocaine (Carey et al., 2002, 2004; Chen and Reith, 1995; De La Garza and Cunningham, 2000; Herges and Taylor, 1998; Müller et al., 2003; Schenk et al., 1991). Lastly, the highest dose of 8-OH-DPAT has been shown to inhibit locomotor activity (Przegalinski and Filip, 1997), but this was true only when it was co-administered with cocaine. Thus, at this point, we speculate that the differences in locomotor activity on retest reflect distinct underlying neural adaptations that may be pre-existing and relate to findings from rats with a history of repeated drug exposure. This hypothesis is based on findings that behavioral sensitization is evident in LCRs,

while HCRs show a lack of sensitization (Sabeti et al., 2003; unpublished observations). Interestingly, Ben-Shahar et al. (2005) reported differential behavioral responses to the locomotor stimulating effects of cocaine in animals who were previously given short or extended access to self-administer cocaine. Specifically, they reported sensitization in rats with short access compared to tolerance in those with extended access. This escalation model of repeated drug self-administration is associated with addictive behaviors shown in humans (Ahmed and Koob, 1998; Ben-Shahar et al., 2004, 2005).

In summary, we found that two traits previously associated with those who abuse cocaine and other drugs, namely initial sensitivity to drug-induced behavior and impulsivity, are closely related to one another in a rat model of these behaviors. These findings highlight the importance of studying individual differences, especially in the context of pre-disposing traits that overlap with other behaviors that can potentially be related to the vulnerability for drug dependence. It is possible that the disparate drug-induced locomotor responses and ensuing differences in delay-discounting behavior are the result of distinct underlying neural mechanisms. Future studies should focus on how pre-existing traits interact with drug-induced neuroadaptations, as they have the potential to provide a better understanding of the underlying neurobiological mechanisms contributing to individual differences in drug abuse potential. It will also be important to determine how differential sensitivity to cocaine predicts behavior in other experimental paradigms that assess other aspects of the multifaceted trait of impulsivity, such as the five-choice serial reaction time task, differential reinforcement of low rate of responding, or fixed consecutive number schedule.

CHAPTER 4: DIFFERENTIAL VULNERABILITY TO AMPHETAMINE-INDUCED DEFICITS IN A WORKING MEMORY TASK: THE EFFECTS OF EXPOSURE METHOD, AGE, AND INITIAL PSYCHOSTIMULANT SENSITIVITY[3]

Abstract

While amphetamine (AMPH) exposure is known to cause long-term changes in drug-induced locomotor behavior (i.e., sensitization), the direct relationship between cognitive dysfunction and chronic psychostimulant abuse is unclear. Since recreational, and more recently therapeutic, use of AMPH is often initiated during adolescence, this period of dramatic structural and functional development may be linked to an increased predisposition to drug-induced deficits. In addition, pre-existing differences in the acute response of a psychostimulant's behavioral effects may be intensified by subsequent drug exposure and predictive of long-lasting changes in cognitive function. To address these issues, we first classified adolescent and adult male Sprague-Dawley rats as low or high cocaine responders (LCRs and HCRs, respectively) based on their acute locomotor response to 10 mg/kg (i.p.) cocaine in an open-field arena. The rats were then exposed to either saline or AMPH using an intermittent (INT) or escalation-binge (E-B) exposure method, which have been reported to produce distinctive behavioral profiles of sensitization and tolerance, respectively. Rats in the INT group were given saline or 3 mg/kg AMPH (i.p.) every other day for 19 days; those in the E-B groups were given saline or escalating doses of AMPH (1-8 mg/kg, i.p.) over 4 days, followed by a single binge treatment (saline or 8 mg/kg AMPH, every 2 hr for 8 hr). Adolescent rats

[3] Some data from this chapter appeared in the journal Behavioral Brain Research as Sherrill LK, Stanis JJ, Gulley JM (2013) Age-dependent effects of repeated amphetamine exposure on working memory in rats. Jan 3; 242C:84-94. Data are included with the permission of the publisher and is available from http://www.sciencedirect.com and using DOI: 10.1016/j.bbr.2012.12.044

began these treatments on postnatal day (PND) 37 or 41, whereas adult rats began these treatments on PND 92 or 96. After all rats reached adulthood (PND 90 or 123), cognitive performance was assessed using an operant-based delayed matching-to-position (DMTP) and delayed non-matching-to-position (DNMTP) task that is sensitive to prefrontal cortex function. We subsequently measured the effects of acute AMPH (0.3-1.25 mg/kg) and the NMDA receptor antagonist, ketamine (5-10 mg/kg), on DNMTP performance. Lastly, we tested for long-lasting effects of pretreatment by giving dose-appropriate AMPH challenges one week after the final DNMTP session. Repeated AMPH in the INT groups produced sensitization to the drug's stereotypy-inducing effects, and this effect was less pronounced in adolescent- compared to adult-treated rats. Similarly, adolescents in the E-B group were less sensitive to the stereotypy-inducing effects of 8 mg/kg AMPH than adults. However, adolescents were vulnerable to exposure-induced learning and memory impairments, as both INT and E-B rats required more sessions to reach criterion at longer delays and showed delay-dependent deficits. In contrast, only adult-exposed E-B rats showed delay-dependent deficits that persisted through criterion, overtraining, and task reversal (along with adolescent-exposed rats in the intermittent treatment group). Moreover, rats classified as LCRs exhibited the greatest delay-dependent impairments. While AMPH dose-dependently altered DNMTP accuracy in both age-exposed groups, ketamine induced delay-independent effects. The AMPH challenges, given approximately two months after the final pretreatment injection revealed that sensitization was still evident in all rats. Our results suggest that AMPH exposure has long-lasting consequences on learning and memory processes. These deficits are dependent on both the age and

method of exposure, as well as individual differences that are related to the disparate function of dopamine transporters.

Introduction

AMPH is prescribed at an increasingly alarming rate for therapeutic purposes, and is one of the most abused psychoactive drugs in the world (World Drug Report, 2012). Both clinical and preclinical studies have revealed that chronic AMPH use is associated with deficits in concentration and attentional control, decision-making and planning, as well as working memory (e.g., Bechara et al., 1994; McKetin and Mattick, 1998; Rogers et al., 1999; Ornstein et al., 2000; Ersche et al., 2006). Such impairments in functions are proposed to be mediated by the prefrontal cortex and associated limbic circuitry (see Jentsch and Taylor, 1999; Goldstein and Volkow, 2002; Arnsten and Rubia, 2012). While we hypothesize that drug-induced changes in brain function are a major contributing factor to the behaviors that characterize drug use and dependence, results from human studies make it rather difficult to ascertain whether co-morbid personality traits like impulsivity and cognitive dysfunction predate the onset of drug use or occur as a consequence of chronic use. Accumulating evidence from animal studies is incongruent. A few studies suggest that repeated exposure to AMPH leads to chronic deficits (Castner et al., 2005), while more report that AMPH exposure did not lead to impairments in working memory (Featherstone et al., 2008), spatial learning or memory (Russig et al., 2003), or delayed alternation (Stefani and Moghaddam, 2002).

Previously, we showed that adaptations in the locomotor response to AMPH can be dissociated from drug-induced adaptations in cognitive behavior when an intermittent treatment method of AMPH exposure is utilized (Stanis et al., 2008b). This is important because numerous studies have demonstrated that intermittent treatment with psychostimulants like AMPH leads to plasticity in the mPFC (Robinson and Kolb, 2004; Kalivas, 2007) and these changes have been hypothesized as important substrates for the long-lasting adaptations in behavior observed in human psychostimulant abusers (Everitt and Wolf, 2002; Robbins and Everitt, 2002; Jones and Bonci, 2005; Kauer and Malenka, 2007). Thus, the method, or pattern, of drug exposure is an important factor to consider. An AMPH escalation-binge exposure method (adapted from Segal and Kuczenski, 1997) more closely approximates the pattern of use by human AMPH abusers and might contribute to the development of AMPH-induced adaptations in cognitive behavior. Escalated self-administration of cocaine has been shown to produce locomotor tolerance to its stimulant effects on locomotor activity (Ben-Shahar et al., 2005), in contrast to the vast studies showing behavioral sensitization. Interestingly, cognitive deficits have been reported in rats trained to self-administer cocaine, but only in those found to escalate their intake over time (George et al., 2008). Thus, dose escalation should not be overlooked in treatment paradigms, and several studies underscore a translational relevance (Segal et al., 2003; O'Neil et al., 2006; Kuczenski et al., 2007; Belcher et al., 2008; Izquierdo et al., 2010).

Another factor that might contribute to AMPH's ability to induce cognitive dysfunction is the age at which exposure occurs. AMPH use typically starts during adolescence (DeWit et al., 1997), when the prefrontal cortex is going through normal

development changes (Cunningham et al., 2002; Casey et al., 2005; Markham et al., 2007). Early exposure to AMPH could lead to deleterious effects in the later expression of behavior and cognitive function. One study showed that even AMPH exposure given repeatedly at low doses produced changes in spine density in the mPFC (Diaz Heijtz et al., 2005). Unfortunately, they only reported evidence on the structural organization and not on any behavioral correlates. Several reviews have outlined the importance of studying adolescence at the behavioral (Laviola et al., 1999; Spear, 2000; Laviola et al., 2003) and neural systems (Andersen, 2003; Ernst and Fudge, 2009; Brenhouse and Andersen, 2011) levels.

A third factor that might overlap with AMPH-induced changes is the initial sensitivity to a psychostimulant. In humans, individual differences in sensitivity predicted greater use and dependence (Davidson et al., 1993; Lambert et al., 2006). Pre-existing differences in the acute response of a psychostimulant's behavioral effects may be intensified by subsequent drug exposure and predictive of long-lasting changes in cognitive function. It has been shown previously that rats classified by a single injection of cocaine can be a useful model for studying individual differences. A number of studies have shown that the cocaine-induced phenotype can be used to predict a number of behaviors, such as conditioned place preference (Allen et al., 2007), intravenous cocaine self-administration (Mandt et al., 2009), drug-discrimination (Klein and Gulley, 2009), and impulsive choice behavior (Stanis et al., 2008a). The primary target underlying the individual differences is the function of dopamine transporters (DATs) in the dorsal striatum and nucleus accumbens (Sabeti et al., 2002, 2003) and not to pharmacokinetic factors (Gulley et al., 2003). While no studies have addressed

neural changes outside of the dorsal and ventral striatum, given the extensive behavioral changes, it is not implausible to assume parallel changes in other neural systems, like the prefrontal cortex.

The primary aim of the current study was to look at the long-lasting effects of repeated AMPH exposure (intermittent and escalation-binge schedules) on locomotion and cognitive function in subjects exposed during adolescence or adulthood. This is important because very few studies carefully examine both the locomotor profiles and cognitive behavior in the same animals after chronic drug treatment. In addition, by classifying rats based on their initial sensitivity to a psychostimulant, we wanted to examine whether the acute response could be predictive of any lasting cognitive dysfunction. Working memory was assessed using a delayed matching-to-position (DMTP) task has been shown consistently to be highly sensitive to changes in mPFC function (Fuster and Alexander, 1971; Kolb, 1990; Goldman-Rakic, 1995; Dias and Aggleton, 2000; Brown and Bowman, 2002). After rats learned the task, we also examined reversal learning by switching to a delayed non-matching-to-position (DNMTP) task and then examined whether acute treatment with AMPH or ketamine, an NMDA antagonist, altered memory performance. Lastly, all rats were given a challenge injection of AMPH (intermittent: 3.0 mg/kg; escalation-binge: 8.0 mg/kg) to determine if long-lasting changes in locomotor activity were evident.

Methods

Subjects. The adult-exposed males (n=55) were Sprague-Dawley rats obtained from Harlan (Indianapolis, IN, USA) on postnatal day (PND) 75 and housed individually

upon arrival. The adolescent-exposed rats (n=50) were bred in our animal facility from stock rats obtained from Harlan. Males were group-housed (2-3 per cage) following weaning at PND 25 and were housed individually after PND 85. They were maintained on a 12:12 hr light/dark cycle (lights on at 08:00) with experimental sessions conducted between 08:30 and 19:30 hr. Rats were handled three times at 15-min intervals prior to being used in experiments (PND 32-34). Food was available ad libitum before the rats were housed separately and again when they completed operant training and testing. During operant training and testing, rats were food restricted to approximately 85% of their free feeding weight. Water was always available ad libitum. All experimental procedures were approved by the IACUC at the University of Illinois, Urbana-Champaign and were consistent with the *Principles of Laboratory Animal Care.*

Apparatus. Locomotor activity was measured in an open-field chamber that consisted of a transparent, Plexiglas box (40.6 x 40.6 x 40.6 cm) surrounded by photobeams (Coulbourn Instruments; Allentown, PA). Each apparatus was connected to a computer operating software (TruScan, v. 2.01; Coulbourn Instruments) that recorded all horizontal and vertical beam breaks (100 ms sampling rate). The horizontal beam breaks, or coordinate changes, were converted into distance traveled (cm). The chambers were individually contained inside sound-attenuating cubicles (76 x 80 x 63 cm). Each cubicle contained a speaker (76 mm dia.) fixed to one wall, two ceiling-mounted white lights (4 W) for dim illumination, and a ceiling-mounted camera between the two lights. White nose (70 dB) was played continuously through the speakers when rats were in the testing room.

Operant behavior was monitored in standard operant chambers (Coulbourn Instruments). One wall of the chamber contained two retractable levers that were positioned on either side of a centrally located food trough. Infrared detectors were used to monitor head entries into the food trough. White cue lights were located above each lever. A white houselight was located near the top of the chamber on the opposite wall.

Initial behavioral characterization: Locomotor activity in the open-field. Rats were first tested in the open-field on PND35 or PND90. After a 30-min acclimation period in the testing room, rats were placed in the open-field chambers for 90 min. They were then removed from the chamber, injected (i.p.) with (-) cocaine HCl (10 mg/kg) and placed back into the chamber for an additional 60 min. This dose was chosen based on previous studies (Sabeti et al, 2002; Gulley et al, 2003; Stanis et al., 2008a) showing that it is optimal for inducing the widest range of behavioral responses in male Sprague-Dawley rats. After the testing session, rats were returned to the colony room and given one day off before being randomly assigned to one of six repeated exposure groups.

Repeated saline or AMPH treatment. Rats were randomly assigned to one of six groups that received saline or AMPH on an intermittent or escalation-binge schedule during adolescence or adulthood. Rats assigned to the intermittent group were given once daily injections (i.p.) of 0.9% saline or 3 mg/kg AMPH, every other day for 20 days (10 injections total). We previously used this procedure to induce long-lasting behavioral sensitization (>3 months) in rats exposed during adulthood (Stanis et al., 2008b). Injections for the intermittent group began on PND37 (n=8 given saline; n=16 given AMPH) or PND92 (n=8 given saline; n=19 given AMPH) and ended on PND55 or

PND110. Rats assigned to the escalation-binge group were given three daily injections of 0.9% saline or AMPH (i.p.), with the administered AMPH dose increasing progressively over four days. Rats started with 1 mg/kg on treatment day 1 and ended on treatment day 4 with 8 mg/kg AMPH. After a day of no injections, they were given an AMPH "binge" where injections of 8 mg/kg were given every 2 hours for a total of 4 injections (see Table 4.1 for summary). Two days later, rats were given an initial challenge injection of saline or 8 mg/kg AMPH. Injections for rats in the escalation-binge treatment group began on PND41 (n=8 given saline; n=18 given AMPH) or PND96 (n=7 given saline; n=21 given AMPH) and ended on PND49 or PND104. All injections were either given in an open-field chamber, a testing cage, or the rat's home cage. After treatment ended, rats remained in their home cage assignment until PND85 or PND118, when food restriction and operant training began.

DMTP/DNMTP behavior. All rats were food deprived (~85%) over a period of 5 days and then began lever press training on a continuous fixed reinforcement schedule. Training on an operant version of the delayed matching to position (DMTP) task (adapted from Dunnett, 1985) first consisted of session without delays. During the no delay, daily sessions, each of the 100 trials began with a 5-s ITI with the house light on. Lever A or Lever B was randomly presented, with equal probability, and the rat had 10 s to press the presented lever or else the trial ended and the lever retracted, the house light turned off, and a new trial began. Upon a successful lever press, the lever would retract and the magazine light turned on to cue the rat to the food trough. After a nose poke into the trough, the previously presented lever is presented again, and the rat received a food pellet reinforcement if the same lever was pressed. In this step, the rat

had no choice after nose poking but to choose the sample lever because only the sample lever was presented. In the next daily session, the nose poke led to both levers being extended. Each session in this stage and the following stages contained 112 trials. Now, the rat must select the lever that was originally presented in order to receive a reward. Responses on the opposite lever were not reinforced and instead resulted in a 5-sec timeout period. Once the rat achieved 85% correct or better performance on consecutive sessions, the next phase of training began. During the next phase, delays were introduced between the nose poke after the “sample” lever and the choice presentation of the levers. This essentially forced the rat to remember which lever was presented earlier in order to receive food reinforcement. Delay intervals were randomly selected on each trial; the first set of delays were short (consisting of 0, 1, 2, 3, 4, 5, and 6s). Performance of 85% correct or greater on consecutive sessions allowed the progression onto the next delay block of medium length (0, 1, 2, 4, 8, 12, and 16s), after which the final set of long delays was introduced (0, 2, 4, 8, 12, 18, and 24s). This delay progression was chosen to avoid response extinction, because longer delays have the potential to disrupt performance if the task has not been acquired. Upon reaching performance of 85% correct or higher for consecutive sessions, rats were overtrained for 5 additional sessions. All rats were then switched to the opposite non-matching rule (delayed non-matching to position). This final reversal phase was identical to the DMTP version, except the rewarded lever became the opposite of the sample. This step, along with repeated nosepoke responses during delay phases, alleviated the adoption of positional mediating strategies.

Following the last training session (second session of 85% correct or higher), the effects of AMPH and ketamine on task behavior were assessed in two testing blocks. For the first testing block, rats were given injections of vehicle (saline; 1 ml/kg, i.p.) or AMPH (0.3, 0.75, and 1.25 mg/kg, i.p.) 5 min before they were placed in the operant chamber for a test session. Injections were given over 5 consecutive sessions (i.e., SDDDS, where S = saline and D = drug). The order of drug doses was chosen based on a Latin square design, with a particular order assigned to each rat randomly. Rats were given a day off before starting the second block of injections. For these tests, ketamine (5, 7.5, 10 mg/kg, i.p.) was given 10 min prior to placement in the operant chambers.

Open-field AMPH challenge. After their final operant session (> PND240), rats were given access to food ad libitum. One week later, they were challenged with the dose respective to their original treatment group. Thus, rats in the intermittent group were given a 3.0 mg/kg i.p. AMPH injection, while rats in the escalation-binge group were given an 8.0 mg/kg AMPH injection. The saline rats were equally divided between groups and given either 3.0 or 8.0 mg/kg AMPH.

Drugs. The (-) cocaine HCl used in this study was obtained from NIDA (RTI International; Research Triangle Park, NC). *d*-Amphetamine sulfate was obtained from Sigma-Aldrich (St. Louis, MO). Ketamine HCl was obtained in a 100 mg/ml injectable solution (Ketaset; Pfizer Animal Health; Fort Dodge, IA) and diluted with sterile saline to appropriate concentrations for injections. Cocaine and AMPH were dissolved in sterile saline (0.9% NaCl), with dosages calculated based on the weight of the salt. All injections were given at a volume of 1 ml/kg.

Data analyses. All analyses were conducted using SAS 9.3 (SAS Institute, Inc.; Cary, NC) and SQL Server 2005 Developer (Microsoft; Seattle, WA). All graphs show group means ± SEM. Cocaine-induced locomotor activity in the open-field was assessed by determining the cumulative activity during the first 30-min following injection. Here and as in previous studies (Gulley et al., 2003; Gulley, 2007; Stanis et al., 2008a), rats with activity scores in the lower half of the population distribution were designated LCRs and those in the upper half were designated HCRs. LCRs and HCRs were assigned to treatment groups to ensure an equal representation across groups. The statistical significance of group differences in cocaine-induced activity was determined using two-way ANOVA (class x age).

For analysis of locomotor behavior in the intermittent and escalation-binge groups, two methods were used: one quantitative and one semi-quantitative. Ambulatory distance was quantitatively measured by photobeam breaks and was recorded as the total horizontal distance traveled minus periods absent of locomotion when the animal exhibited a repetitive behavior. In order to accurately measure the range of behaviors, stereotypy was semi-quantitatively measured for 30 second intervals every 5 minutes by a blind scorer from recorded videos. Stereotypy intensity was rated as mild (1), moderate (2), or intense (3) and multiplied by the duration (seconds spent exhibiting the scored behavior), giving a scoring range from 0-90. Because a time-course of AMPH-induced behavior (for ambulation and stereotypy) for the intermittent schedule in adults was previously published (Stanis et al., 2008b), ambulation data were summed over the 60 min session, while stereotypy data were averaged. Data were analyzed using a repeated two-way ANOVA, with treatment day

as within-subjects factor and treatment group as the between-subjects factor. Data from the escalation-binge groups were analyzed using a repeated two-way ANOVA (binge-run: treatment group x time) or three-way ANOVA (treatment group x session x time). Follow-up analyses of significant interactions were performed with one- or two-way ANOVA and Tukey post hoc tests. Missing values were excluded from analyses.

For analyses of operant-task behavior, several measures were used. The progression of task performance was monitored by the number of sessions required to reach criterion at each of the delay blocks. Working memory was assessed by calculating the mean percentage of correct choices across delays for the long delay-block sessions. In order to examine delay-dependent changes over time, percent correct was compared for the first two sessions in the long delay-block and for the first two sessions a rat met performance criterion. In order to figure out an animal's accuracy between two levers, index Y was also calculated according the following formula;

$$\text{Index Y} = \left| \frac{p(\text{cor} \mid A) - p(\text{cor} \mid B)}{p(\text{cor} \mid A) + p(\text{cor} \mid B)} \right|$$

where p (cor | A/B) is the probability of making a correct response when the sample was presented as lever A or B, respectively. Data were analyzed with repeated-measures ANOVAs (treatment group as the between-subjects factor and delay and delay block as within-subjects factors). When appropriate, Tukey post hoc tests were used.

Performance following AMPH and ketamine drug challenges was assessed by calculating mean percent correct and the number of trials omitted during each session. These measures were analyzed with repeated-measures ANOVAs with treatment group

as the between-subjects factor, and dose as the within-subjects factor. Tukey post-hoc tests were used. In all tests, *p* values less than 0.05 were considered significant.

Results

Locomotor behavior

In both adolescent (PND35) and adult rats (PND90), the first exposure to 10 mg/kg cocaine resulted in disparate locomotor activity that could be described by separating rats into LCRs and HCRs (Fig. 4.1). This difference was statistically significant [main effect of class: $F(1,101) = 158$, $p<0.001$]. Adolescent LCRs exhibited on average 66% less cocaine-induced activity compared to that observed in HCRs, whereas adult LCRs showed 50% less cocaine-induced activity compared to HCRs. In general, adolescent rats showed 35% less ambulation than adults [main effect of age: $F(1,101) = 46.1$, $p<0.001$].

As shown in Fig. 4.2, AMPH produced significant changes in ambulation and stereotyped behavior following the first (T1) and tenth (T10) injections. These changes in activity were also evident when an AMPH challenge was given at the conclusion of operant testing, which occurred approximately four months after pre-treatment for rats exposed during adolescence and three months for rats in the adult-exposed group. Separate two-way ANOVAs revealed significant interactions between group and treatment session for ambulation [$F(6,92) = 22.2$, $p<0.001$] and stereotypy [$F(6,93) = 7.69$, $p<0.001$]. Compared to saline-treated controls, rats in both the adolescent- and adult-exposed groups exhibited significant increases in ambulation and stereotypy the first and tenth injections with 3 mg/kg AMPH. For adolescent-exposed rats, ambulation

was elevated in a similar magnitude following the first and tenth injections. However, in adult-exposed rats, there was a significant reduction in ambulation following the tenth injection compared to the first. All rats received an injection of 3 mg/kg AMPH during the challenge session. Compared to saline pre-treated controls, adolescent- and adult-exposed rats displayed less ambulation but greater stereotyped behavior. Rats in the saline pre-treated groups exhibited significant increases in both ambulation and stereotypy compared to their last saline session, and these effects were only significantly different from stereotyped behavior observed in adult-exposed rats following their first AMPH injection (Fig. 4.2B). Furthermore, both AMPH-exposed groups showed enhanced stereotypy compared to their last treatment session (T10), but the overall magnitude of the stereotypy response was lower in adolescent-exposed rats. Pearson correlation analysis did not reveal any significant relationship between distance traveled after cocaine injection and the response to AMPH injections.

AMPH escalation followed by a high-dose, binge AMPH produced episodes of intense, focused stereotypy. As shown in Fig. 4.3, ambulatory activity was measured during the binge session, where four injections were given, once every two hours. After a maximal level of stereotyped behavior was observed, the amount of ambulation increased. A two-way repeated measures ANOVA revealed a significant interaction between treatment group and time [$F(93,1271) = 2.86$, $p<0.001$]. During the last 30 minutes of each binge injection, adolescent-exposed rats showed a significant increase in ambulation compared to adult-exposed rats. In addition, rats in the adolescent-exposed group showed maximal ambulation during the final 15 minutes (at the 480 min mark), compared to the previous three injections (Fig. 4.3).

After the binge session, rats were given one day off before a challenge injection was administered in the open-field (8 mg/kg AMPH). As shown in Fig. 4.4, a high dose injection produced significant changes in ambulation and stereotypy. Separate three-way ANOVAs revealed significant interactions between group, treatment session, and time for ambulation [group x treatment session: $F(3,1552) = 82.6$, $p<0.001$; three-way: $F(45,1552) = 2.41$, $p<0.001$] and for stereotypy [group x treatment session: $F(3,912) = 75.9$, $p<0.001$; three-way: $F(45,912) = 1.57$, $p=0.076$]. While the pattern of ambulation was very similar between age groups, adolescents showed a rapid decrease starting 180 minutes post-injection (Fig. 4.4A). This decrease was associated with a rapid decrease in stereotypy (Fig. 4.4B). After the final challenge, which occurred approximately three months after the last injection, ambulation was greatly reduced in both age-exposed groups, compared to the first challenge. Once again, the reduction in ambulation was associated with an increase in intense, focused stereotypy over a much longer time span. While the stereotypy response was almost identical during the final challenge session, compared to the first challenge, the response in adolescent-exposed rats was enhanced to a greater extent.

Delayed Matching to Position Behavior

Prior exposure to AMPH altered DMTP behavior (Fig. 4.5-4.7). While all rats learned the task, some rats required more sessions to reach criterion (Fig. 4.5). Only adult-exposed rats in the escalating-binge group showed impairments in acquiring the knowledge of how to perform the task (matching without delays; Fig. 4.5B). After delays were introduced, adolescent-exposed rats in the intermittent group and adult-

exposed rats in the escalating-binge group were most affected during the long delay and reversal (DNMTP) blocks. That is, the groups took longer (needed more sessions) than saline-treated animals to reach criterion. As shown in Fig. 4.5, the deficits were dependent on treatment group [$F(5,99) = 8.45$, $p<0.001$] and training phase [$F(4,394) = 115$, $p<0.001$]. The interaction between group and phase was not significant ($p>0.05$). Pearson correlation analysis did not reveal any significant relationship between distance traveled after cocaine injection and learning the task rules.

Significant delay-dependent impairments in DMTP behavior were observed in both adolescent- and adult-exposed rats during the first two sessions of the long delay block (Fig. 4.6 A-B). Two-way repeated measures ANOVA revealed significant main effects of treatment group [$F(5,99) = 10.6$, $p<0.001$] and delay [$F(6,594) = 211$, $p<0.001$], with a significant interaction [$F(30,594) = 8.45$, $p<0.05$]. More deficits were observed in the adolescent-treated rats, specifically at 8 and 12 s in the escalation-binge treatment group, and at 8, 12, 18, and 24 s in the intermittent treatment group, when compared to saline-treated rats. In contrast, the escalation-binge adult-exposed rats showed significant impairments at 12, 18, and 24 s; whereas, the intermittent group only showed a significant difference at 18 s. Working memory performance was markedly improved during the two sessions when criterion was met (Fig. 4.6 C-D). Two-way repeated measures ANOVA revealed significant main effects of treatment group [$F(5,99) = 3.76$, $p<0.01$] and delay [$F(6,594) = 297$, $p<0.001$], but no significant interaction ($p>0.05$). Deficits persisted in the intermittent, adolescent-exposed group (24 s), and in the escalation-binge, adult-exposed group (18 and 24 s).

When working memory performance data were analyzed according to their cocaine response groups, the deficits in both adolescent- and adult-exposed groups were largely found in LCRs (Fig. 4.7). Separate two-way repeated measures ANOVAs revealed significant main effects of treatment group [LCRs: $F(5,44) = 9.43$, $p<0.001$; HCRs: $F(5,49) = 5.37$, $p<0.001$] and delay [LCRs: $F(6,264) = 113$, $p<0.001$; HCRs: $F(6,294) = 97.0$, $p<0.001$], with a significant interaction for LCRs [$F(30,264) = 1.49$, $p<0.05$] and not HCRs ($p>0.05$). LCRs in the intermittent, adolescent-exposed group showed the most deficits, ranging from 4-24 s (Fig. 4.7A). Adult-exposed LCRs in the intermittent and escalation-binge groups showed deficits from 12-24 s (Fig. 4.7B). HCRs, on the other hand, regardless of age or method of exposure, showed no delay-dependent impairments compared to saline-treated controls (Fig. 4.7 C-D). While not significant, escalation-binge, adult-exposed rats showed a trend for differences at very short delays (0-8 s) compared to saline controls.

None of the groups differed from each other ($p<0.05$) on measures of general responsivity: latency to respond to the sample lever, number of nosepokes during the delays, number of omissions, or in index y, a measure of positional strategy (data not shown).

When rats met performance criterion on DNMTP, they were tested for their response to acute doses of AMPH or ketamine (data not shown). While AMPH dose-dependently altered DNMTP accuracy in both age-exposed groups [main effect of dose: $F(3, 15) = 22.0$, $p<0.001$], ketamine induced delay-independent effects (NS main effect of dose, $p>0.05$). Neither drug was found to alter performance between groups (NS main effect of treatment group, $p>0.05$).

Discussion

Chronic AMPH exposure has long-lasting effects on locomotor behavior and cognitive function that are dependent on both age and method of drug exposure. Here, we report evidence for a dissociation between the robustness of the locomotor response after repeated AMPH exposure and ensuing cognitive deficits. That is, while adolescent rats were less sensitive to the stereotypy-inducing effects of both an intermittent and escalation-binge method, they were more vulnerable to exposure-induced learning and memory impairments. Only adolescent-exposed rats in the intermittent treatment group and adult-exposed rats in the escalation-binge treatment group showed delay-dependent deficits that persisted through criterion, overtraining, and task reversal. Moreover, the delay dependent deficits in adolescent- and adult-exposed rats could be predicted based on their initial sensitivity to a single injection of cocaine. When rats were given an AMPH challenge after the conclusion of their final DNMTP session—a procedure that occurred approximately three to four months after their last injection—the sensitization to AMPH's stereotypy-inducing effects was still evident and even enhanced in both adolescent- and adult-exposed rats.

After the initial injection of 3.0 mg/kg AMPH, adolescents and adults responded similarly in both measures of locomotion and stereotypy. This is consistent with previous studies showing no age-dependent differences in the acute response to AMPH (Adriani et al., 1998; Mathews and McCormick, 2007). After the tenth injection of 3.0 mg/kg AMPH, a disparate pattern emerged between age groups in stereotypy, such that adults displayed more intense stereotypy. Adolescents did show sensitization to the

stereotypy-inducing effects across repeated treatments, but the increase was minor compared to adults. The emergence of reduced sensitivity to repeated AMPH in adolescent-exposed, compared to adult-exposed rats, is consistent with prior reports (Bolanos et al., 1998; Richetto et al., 2013). Locomotor sensitization was not observed in either groups, and in fact, adults showed a decrease in ambulation after the tenth injection. After the final AMPH challenge, which occurred approximately three months after the last injection, sensitization to the stereotypy-inducing effects was still observed in both age groups, although the adult-exposed group showed more intense, focused stereotypy.

With respect to the long-lasting sensitization that was observed, it should be noted that these rats received additional injections of AMPH (total cumulative dose of 2.3 mg/kg) and ketamine (total cumulative dose 22.5 mg/kg) across five days of drug and vehicle testing in the DNMTP task. However, the saline controls also had this same level of exposure and their responses to the AMPH challenge were similar to the response seen in AMPH-exposed rats after their first drug exposure. Thus, the drug challenges during the working memory task are unlikely to account for the persistence of sensitization. Similar long-lasting, stimulant-induced behavior has been observed before in adults (Paulson et al., 1991; Stanis et al., 2008b), and in adolescent-exposed animals (Richetto et al., 2013).

A similar pattern of subsensitivity to the stereotypy-inducing effects emerged in adolescent-exposed rats in the escalating dose-binge group. That is, during a binge session with four injections (each 8 mg/kg AMPH) spaced two hours apart, after each injection, adolescents spent less time in stereotypy and more time ambulating. These

results agree with the only study we know of looking at escalating dose-binge but with cocaine (Caster et al., 2005). Two days following the binge session, rats in the AMPH treatment groups were given a challenge injection of 8 mg/kg AMPH and behavior was monitored for four hours. While the pattern of ambulation was very similar between age groups, adolescents emerged from intense stereotypy much faster than adults. After the final challenge, which occurred approximately three months after the last injection, sensitization to the stereotypy-inducing effects was observed in both age-exposed groups. Interestingly, the sensitization was more pronounced in the adolescent-exposed group. To our knowledge, no published studies have described the long-lasting effects of an escalating dose-binge model of AMPH exposure in adolescent and adult animals. Taken together with the intermittent treatment groups, different patterns of sensitization suggest that the neuroadaptations induced by chronic AMPH may be unique in adolescents relative to adults.

We observed age- and method of exposure-dependent differences on learning and working memory function. Rats exposed to 3 mg/kg AMPH during adolescence, but not those exposed during adulthood, showed delay-dependent deficits in matching during DMTP training. The working memory deficits were also accompanied by learning impairments, as the same rats required more sessions to reach criterion. Interestingly, rats exposed to an escalating-binge schedule of AMPH during adulthood, but not those exposed during adolescence, showed the same pattern of learning and memory deficiencies. The observed DMTP deficits are likely the result of distinct neural mechanisms, as adolescent-exposed rats showed improvement of memory function, unlike the adult-exposed rats. Thus, we conclude that AMPH caused disruptions in the

normal development of the PFC and its dense connections with the hippocampal formation for adolescents, whereas adults might be more prone to the neurotoxic effects of high-dose AMPH treatment. Degeneration of pyramidal neurons and interneurons in the prefrontal cortex and hippocampus has previously been reported in adult rats exposed to escalating dose-multiple binge methamphetamine (Kuczenski et al., 2007).

After rats completed the matching portion (DMTP), we reversed the rules from matching to non-matching in order to examine the effects on inhibition control. It has previously been noted that rats have an innate and preferred strategy to alternate responses spontaneously (Richman and Dember, 1986), as is the rule with non-matching in the DNMTP task. Interestingly, we found that rats in both intermittent- and escalation-binge-exposed groups, regardless of age of exposure, took longer to learn the specific rules during the DNMTP phase than during the DMTP phases. This finding is counter to the innate bias, even when working memory requirements were identical. Nonetheless, the reversal deficits were most pronounced in the adolescent-exposed intermittent treatment and adult-exposed escalation-binge groups and provides evidence for a lack of inhibition of a learned response, which has previously been shown in animals with selective mPFC damage on a variety of different spatial tasks (Granon et al., 1994; Seamans et al., 1995; Ragozzino et al., 1999; Dias and Aggleton, 2000; Delatour and Gisquet-Verrier, 2000), in humans with PFC damage and impairments in the Wisconsin Card Sorting Test (Berman and Weinberger, 1990; Stuss et al., 2000), and following repeated psychostimulant treatment (Schoenbaum et al., 2004; Fletcher et al., 2005; Izquierdo et al., 2010).

No significant relationship was found between the initial locomotor response to a low dose of cocaine (10 mg/kg) and subsequent locomotor responses to repeated AMPH with either exposure method. While this was somewhat unexpected based on previous results suggesting a positive correlation (Sabeti et al., 2003; unpublished observations), we expect that the differences are likely a result of administering doses that result in intense, focused stereotypy instead of a primary increase in locomotor activity. Fortunately, the initial sensitivity to cocaine could predict certain aspects of delay-dependent performance in the DMTP task. Drug-exposed LCRs were more impaired in a delay-dependent manner than HCRs, regardless of age of exposure and treatment group. Because the DAT is a primary pharmacological target of AMPH, and changes in DA signaling are important for working memory function (Zahrt et al., 1997; Vijayraghavan et al., 2007), the differential effects are possibly a reflection of known differences in DAT function between the phenotypes (Sabeti et al., 2003; Mandt and Zahniser, 2009). However, after repeated cocaine exposure, it has been shown that differences in DAT function disappear, at least in the ventral striatum (Sabeti et al., 2003). Thus, the differential effects could be due to other consequences of neuroplasticity, like alterations in more specific dopaminergic and/or glutamatergic signaling in the PFC, as blunted dopaminergic and glutamatergic function are linked with impairments in PFC function (Brozoski et al., 1979; Seamans and Yang, 2004; Floresco and Magyar, 2006). Studies from Arnsten's and Goldman-Rakic's groups (Zahrt et al., 1997; Vijayraghavan et al., 2007) suggest that optimal levels of DA D1 receptor stimulation contribute to the most advantageous cognitive performance, with insufficient or excessive stimulation leading to impaired performance in mPFC-sensitive

tasks. In essence, a normal neuromodulatory drive would lead to a balance of excitation and inhibition and result in normal PFC function, while an altered one would lead to dysfunctional behavioral performance.

In summary, repeated and intermittent exposure to AMPH in adolescence leads to cognitive deficits later in life. This same exposure method does not lead to deficits in adult-treated rats. In contrast, similar deficits are seen in adult-treated, but not adolescent-treated, rats that were exposed to an escalating dose-binge treatment method. These results are the first to report such findings for both intermittent and escalation-binge exposures. Moreover, very few studies carefully examine both the locomotor profiles and cognitive behavior in the same animals. These deficits appear to be closely associated with initial differences in cocaine-induced locomotor activity rather than related to overlapping locomotor changes induced by repeated drug treatment (i.e., sensitization). Understanding the disruptions in plasticity within separate brain regions and targeting of circuit imbalances should play a major role in therapeutic advances in drug use and dependence. With findings of differential vulnerabilities that are age- and pattern of drug exposure-dependent, we predict that these differences are the result of distinct mechanisms within the mesocorticolimbic networks, especially given that cortical and subcortical regions mature at different rates during adolescence (Tau and Peterson, 2010). Further explorations between the functional interactions of multiple systems—namely, the hippocampus, mPFC, and ventral striatum—are warranted in order to better understand the role of development and subsequent neural mechanisms of chronic drug exposure.

CHAPTER 5: ADAPTATIONS IN MEDIAL PREFRONTAL CORTEX FUNCTION ASSOCIATED WITH AMPHETAMINE-INDUCED BEHAVIORAL SENSITIZATION[4]

Abstract

Neuroadaptations in the prefrontal cortex (PFC) are hypothesized to play an important role in the behavioral changes associated with repeated psychostimulant drug exposure. However, studies that measure neuronal activity during drug-induced behavior are few in number, and they have not described how firing activity in the medial PFC (mPFC) is altered during the development and expression of psychostimulant sensitization. To address this, we recorded single neuron activity in the mPFC of male rats (n=9) that were exposed repeatedly to saline or amphetamine (AMPH; 1.0 mg/kg, i.p.) for five days. Daily recordings were obtained in an open-field environment for the five treatment sessions and after two challenge injections (saline or 1.0 mg/kg AMPH) that followed a 3-day withdrawal (or drug-free) period. We found that rats exposed to AMPH developed locomotor sensitization to the drug that emerged on the fifth treatment session and became statistically significant at AMPH challenge. This was associated with no change in baseline (i.e., pre-injection) activity of mPFC neurons across the treatment or challenge sessions. Following the first AMPH injection, mPFC neurons responded primarily with reductions in firing, with the overall pattern and magnitude of responses remaining largely similar following repeated treatment. The exception was in the minority of cells that respond to AMPH with increases in firing rate. In this population, the magnitude of excitations peaked during the fifth AMPH exposure

[4] This chapter appeared in its entirety in the journal Neuroscience as Gulley JM, Stanis JJ (2010) Adaptations in medial prefrontal cortex function associated with amphetamine-induced behavioral sensitization. 166(2):615-24. This article is reprinted with the permission of the publisher and is available from http://www.sciencedirect.com and using DOI: 10.1016/j.neuroscience.2009.12.044

and was still relatively elevated at the AMPH challenge. Furthermore, these units increased firing during a saline challenge that was given to assess associative conditioning. These results suggest that AMPH-induced adaptations in mPFC function are not as apparent as AMPH-induced adaptations in behavior. When mPFC adaptations do occur, they appear limited to the population of neurons that increase their firing in response to AMPH.

Introduction

When psychostimulant drugs are administered repeatedly, they have a significant potential to produce either a reduction (tolerance) or an increase (sensitization) in responsiveness after subsequent exposure to the same or lower doses. In laboratory animals, the most frequently studied effect with drugs such as cocaine and amphetamine (AMPH) is sensitization. In rats and mice, for example, repeated but intermittent exposure to these drugs results in augmentation of species-specific motor behaviors such as locomotion, head movement and sniffing, and stereotyped (i.e., repetitive) head, limb, and orofacial movements (Segal and Schuckit, 1983). The extent and duration of this sensitization is influenced by drug dose, treatment regimen, and environmental context (Robinson and Becker, 1986; Badiani and Robinson, 2004). Sensitization has also been described in humans, especially in relation to AMPH-induced psychosis (Robinson and Berridge, 1993) and AMPH-induced euphoria, drug "liking" and motor activation (Strakowski et al., 1996, 2001; Boileau et al., 2006).

It is hypothesized that these enduring behavioral changes are the result of drug-induced neuroadaptations, particularly within the brain's reward circuitry (Robinson and

Kolb, 2004; Jones and Bonci, 2005; Kauer and Malenka, 2007). For example, increases in apical dendrite length, spine density, and the number of branched spines in the nucleus accumbens (NAc) and prefrontal cortex (PFC) have been demonstrated after both experimenter- (Robinson and Kolb, 1997, 1999; Singer et al., 2009) and self-administered (Robinson et al., 2001; Crombag et al., 2005) cocaine or AMPH. Repeated AMPH exposure has also been shown to increase the number of synapses onto spines in the infralimbic and prelimbic regions of the medial PFC (mPFC; Morshedi et al., 2009). These drug-induced changes have been shown to persist for long periods of time, as much as 3.5 years following AMPH administration in a study of the PFC in non-human primates (Selemon et al., 2007). Repeated exposure to cocaine or AMPH also has been shown to produce long-lasting changes in neuronal excitability and synaptic efficacy in the ventral tegmental area (VTA), NAc, and PFC (Onn and Grace, 2000; Thomas et al., 2001; Dong et al., 2005; Nogueira et al., 2006; Peterson et al., 2006; Kourrich et al., 2007; Chen et al., 2008; Ford et al., 2009).

The relationship between these drug-induced adaptations and the expression of behavioral sensitization is not clear, however, because the aforementioned studies utilized *in vitro* methods that required correlation of the observed anatomical or functional changes with the behavioral responses that animals expressed well before the neuroadaptations were assessed. For example, in the studies of neuronal function, one or several days elapsed between the last drug injection and the measurement of NAc or PFC activity. One method of more directly assessing this relationship is to perform *in vivo* electrophysiology recordings from behaving animals as they undergo repeated drug exposure and subsequent challenge injections. However, in the

comparatively few studies where this was done in rats with a history of cocaine (Stalnaker et al., 2006; Sun and Rebec, 2006) or AMPH (Homayoun and Moghaddam, 2006) exposure, there was no description of neuronal responses as they related to drug-induced behavior and the expression of behavioral sensitization. One noteworthy exception to this is a recent report (Ball et al., 2006) of adaptations in the function of neurons in the dorsal striatum following repeated exposure to (±)3, 4-methylenedioxymethamphetamine (MDMA, or ecstasy). In this study, units recorded from rats that expressed locomotor sensitization to MDMA increased their firing rate to a greater extent following MDMA challenge compared to the first exposure to MDMA.

In the current study, we investigated the relationship between AMPH-induced behavioral sensitization and both baseline and AMPH-induced changes in mPFC activity using *in vivo* electrophysiological recording methods. We focused on the mPFC because a number of previous studies have highlighted its important role for not only the acute locomotor response to AMPH (Dunnett et al. 1984; Bast et al. 2002; Hall et al., 2009), but also the development and expression of locomotor sensitization following repeated AMPH exposure (Wolf et al., 1995; Cador et al., 1999; Bjijou et al. 2002). Recordings were obtained as rats behaved in an open-field arena before and after daily injections of saline or 1.0 mg/kg AMPH, with subsequent analyses performed on data from four recording sessions: treatment day 1, treatment day 5, and two separate challenge injection sessions. During these challenge sessions, which occurred on consecutive days and were preceded by three days during which rats remained in their home cage, rats were given either saline or AMPH to test for evidence of conditioning and locomotor sensitization, respectively.

Methods

Subjects. Male Sprague-Dawley rats (n = 9), bred in our animal facility from stock rats obtained from Harlan (Indianapolis, IN), were housed individually starting at ~2 months of age and were 3-5 months old (375-500g) at the beginning of experiments. They were maintained on a 12:12 h light:dark cycle (lights on at 0800) with food and water available *ad libitum*. Rats were handled five times for 15 min intervals prior to being used in experiments, which were conducted between 0900 and 1800 h. All experimental procedures were approved by the IACUC at the University of Illinois, Urbana-Champaign and were consistent with the *Principles of Laboratory Animal Care* (NIH Publication no. 85-23).

Electrode construction and surgical procedures. Electrode bundles were constructed of 16 or 32 formvar-coated nichrome microwires (25 μm diameter; A-M systems, Sequim, WA) that were cut and assembled into single cylindrical bundles. The bundles were placed in customized stainless steel tubing (22G; Small Parts, Miami Lakes, FL) before being attached with conductive epoxy to 10-pin connectors (Omnetics; Minneapolis, MN). Ground wires, which were constructed from Teflon-coated stainless steel wire (140 μm diameter; A-M systems) with insulation removed at the ends, were attached to a stainless steel machine screw (#0-80; Small Parts) and one channel of the connector. Impedances on recording electrodes were reduced to 0.3-1.0 MΩ by passing current through sterile saline solution ("saline bubbling").

Electrode bundles were implanted unilaterally into the mPFC of rats that were anesthetized with ketamine and xylazine (100 and 10 mg/kg, i.m., respectively, followed by 25 mg/kg ketamine, i.p., boosters as needed). A moisturizing lubricant (Moisture

Eyes PM; Bausch & Lomb, Rochester, NY) was applied to prevent corneal drying. Holes were drilled over the right mPFC (3.0 anterior-posterior and 1.3 medial-lateral to bregma; Paxinos and Watson, 1998) and at several other anterior and posterior skull positions for the insertion of stainless steel screws to anchor dental acrylic and the ground wires. In the hole overlying the mPFC, the dura was reflected with a 27G needle tip and electrode bundles were lowered 4.2 mm dorsal-ventral at a 9.5° angle towards the midline. When necessary, the craniotomy was sealed with silastic material (Kwik-Sil; WPI, Inc., Sarasota, FL) before the application of dental acrylic. An analgesic dose of carprofen (5.0 mg/kg, s.c.) was administered every 6-12 hours for the first day after surgery.

Electrophysiological techniques. Each microwire was connected to one channel of a 32-channel unity gain field effect transistor preamplifier. Signals from this headstage amplifier were transmitted via lightweight cabling through a multi-channel commutator (Plexon Inc.; Dallas, TX) that allowed the rat to move freely in the testing environment. For extracellular unit data, signals were amplified, band-pass filtered (250 Hz to 8 kHz) and digitized (40 kHz sampling rate). Signal acquisition and real-time spike sorting was accomplished via digital signal processors located within a Multichannel Acquisition Processor (MAP; Plexon, Inc.). For open-field chamber recordings, videos were captured and time-stamped (CinePlex; Plexon, Inc.) for later synchronization of behavior with neural activity (e.g., peri-event time histograms, or PETHs).

Spike activity was separated from background on each of the 16 or 32 channels using a combination of manual and semi-automated methods (Sort Client and Offline

Sorter; Plexon, Inc.). Initially, an absolute refractory period of at least 1.1 ms and a waveform amplitude threshold of at least 2.5 standard deviations (SDs) higher than the noise amplitude were used to isolate single units from background; obvious noise artifacts were also removed from the data set. In addition to waveform template matching, clustering algorithms and principle component analysis (PCA) were used to optimize these discriminations (Fig. 5.3A). Spike activity was identified as originating from putative interneurons or pyramidal cells based on well-established electrophysiological criteria (Kim and Conners, 1993; Mainen and Sejnowski, 1996; Jung et al., 1998; Barthó et al., 2004) that included action potential waveform shape and spike duration, mean discharge rates, inter-spike interval histograms, and autocorrelograms. Specifically, single-unit activity was considered to be from putative interneurons if it had a high firing rate that followed a regular pattern, whereas pyramidal neurons were identified by burst firing patterns with peaks at 3-10 ms in their autocorrelograms (if the maximum peak is $\geq$ 50% of the maximum bin value of the first 50 ms; Fig. 5.3B) or regular-spiking with a slow-rising slope in their autocorrelogram (mode of interspike-interval histogram > 35 ms). Because well-separated putative interneurons made up a small sample of recorded cells (~5%), only data from putative pyramidal cells were used for further analyses.

Behavioral testing. Tests of locomotor activity were performed in an open-field arena, which consisted of a vinyl floor (41 cm x 41 cm) and 41 cm high Plexiglas walls. The arena was enclosed in a wooden black cubicle with a black curtain across the front. It was dimly illuminated with a Fiber-Lite illuminator (Dolan-Jenner, Lawrence, MA) that was mounted through holes in the ceiling of the surrounding cubicle. Also mounted to

the cubicle ceiling was a small, high-resolution video camera. An audio speaker was located on the side of the cubicle near the top, and on the opposite side a small LED cue light was centered at the bottom of the arena.

Seven to ten days following surgery, rats were taken from the colony room to the testing room, the headstage was attached to the connectors implanted to their skull, and they were allowed to acclimate in a towel-lined holding pot. On this and subsequent test days, rats underwent two daily recording sessions. In a morning session, which was used to assess the stability of recorded units, rats rested in the holding pot and recordings lasted for ~15 min. An afternoon session was used for testing and lasted for ~1 hr 45 min. It consisted of recording during a 15 min period of rest in the holding pot just outside the open-field arena, a 30 min habituation period inside the open-field arena, a 45 min period in the open-field arena after they had been removed and injected (i.p.) with either 1.0 ml/kg saline or 1.0 mg/kg AMPH, and a 15 min period in the holding pot. Rats were then returned to the colony room.

On Day 1 (saline pre-test), all rats were injected with saline during the afternoon session. On subsequent test days, five rats were given AMPH (AMPH group) and two rats were given saline (saline group) using a protocol we have used previously to demonstrate AMPH-induced locomotor sensitization and conditioned behavior (Hall et al., 2008). On days 2-6 (tests 1-5), rats received either saline alone or AMPH and a compound stimulus (28 V, white LED and a 1 kHz, 55dB tone) that was presented in a 5 sec on/5 sec off pattern for the duration (45 min) of the post-injection recording in the open-field arena. Following a three-day withdrawal period where they remained in the colony room, rats began the first of two daily afternoon sessions with a saline or AMPH

challenge. For the first challenge session, all rats received saline but the AMPH group was presented with the compound stimulus. For the second challenge session, rats in the saline group were given another saline injection whereas those in the AMPH group were given an AMPH challenge (1.0 mg/kg, i.p.). For both groups, the compound stimulus was presented for the duration of the post-injection period.

Histology. Following the last test session, rats were deeply anesthetized with chloral hydrate and marking lesions were produced by passing current (50 μA, cathodal; 5 s) through electrode channels where presumed mPFC neurons were recorded. Rats were then perfused transcardially with 0.9% saline and a solution of 5% potassium ferrocyanide in formalin, which reacted with metal deposits at the lesion site to produce a blue-green stain. Brains were removed and stored in formalin until they were frozen and sectioned (60 μm thickness) on a sliding microtome. A light microscope was used to analyze cresyl violet stained sections for electrode tracks and tip locations. Only data obtained from those electrodes determined to be in the vicinity of neurons in layer V of the prelimbic or infralimbic regions of the mPFC (as defined by Paxinos and Watson, 1998) were used for subsequent analysis. Locations of these recording locations are shown schematically in Fig. 5.1.

Drugs. The *d*-AMPH sulfate used in this study was obtained from Sigma-Aldrich (St. Louis, MO). It was dissolved in sterile saline (0.9% NaCl), with the dose calculated based on the weight of the salt. All injections were given at a volume of 1 ml/kg.

Data analysis. Statistical analyses were conducted using SigmaStat 3.5 (Systat Software, Inc.; San Jose, CA) and *p* values less than 0.05 were considered significant. Locomotor activity before and after injections (saline or AMPH) was quantified by

measurement of quadrant crossings in the open-field and summarized into mean values for the 45-min post-injection time period. These data were analyzed using two-way, mixed factor ANOVA (with session as the repeated factor) followed by Holm-Sidak post-hoc analysis.

Electrophysiological data were imported into NeuroExplorer (NEX Technologies, Littleton, MA) for summary and analysis using similar methods to those we (Gulley et al., 2002, 2004) and others (Homayoun and Moghaddam, 2006) have described previously. Units recorded on different treatment days were treated as independent units, even though the anchoring of the electrode connectors would suggest that the same or similar populations of cells were sampled across days. Analysis of baseline firing was done using the first and second 15-min time intervals of the 30-min pre-injection recording period. These were chosen because the first 15 min represents a period when rats were actively engaging in exploratory behavior. During the second 15 min, rats were relatively inactive, usually only exhibiting brief periods (< 5 s) of sniffing or head movements. Firing rate (spikes/s) was first analyzed using three-way ANOVA (group × session × baseline period) followed by two-way, mixed factor ANOVA (with baseline period as the repeated factor) for each of the treatment and challenge sessions. This was followed by Holm-Sidak post-hoc analysis where appropriate.

Changes in firing rate after saline or AMPH administration were analyzed by first determining if there was a consistent alteration in firing during the post-injection period compared to the baseline period preceding injection. Units were characterized as increased or decreased if the mean firing rate following injection was below or above, respectively, the 99% confidence interval of the baseline firing rate, (1) for at least four

consecutive 5-min bins, or (2) for five of the nine 5-min bins that were recorded post-injection. Those units that did not meet this criterion were classified as not changed. Chi-square ($\chi 2$) tests were used to compare the distribution of these response types between treatment groups and within treatment groups across sessions. Bonferroni correction was used to determine the threshold for statistical significance as a control for Type 1 error introduced by the performance of multiple chi-square tests. Thus, the effective α level for these tests was $p < 0.005$. Mean firing rate for units in these categories was then analyzed using a three-way ANOVA (group × session × response type) followed by two-way ANOVA (group × response type) for each of the treatment and challenge sessions. Post-hoc comparisons were done with Holm-Sidak tests.

Burst firing was assessed using the interval specification algorithm in NeuroExplorer using the following parameters: maximum onset interspike (ISI) interval of 80 ms, minimum offset ISI interval of 160 ms, maximum between-burst interval of 6 msec, minimum burst duration of 10 msec, and a minimum of 3 spikes in a burst). Similar parameters have been used previously in an analysis of cocaine-induced changes in mPFC burst firing (Sun and Rebec, 2006). Analysis of burst rate (bursts/min) during the active and inactive baseline periods was done as described above for firing rate. For analysis of changes in burst activity following saline or AMPH injection, burst rate and % of spikes in bursts during the 5-min period just before injection was compared to that observed following saline or AMPH using a three-way ANOVA (group × session × time period). To address within- and between-group variability in burst activity during baseline and allow for a more direct comparison between groups and across recording sessions (Homayoun and Moghaddam, 2006),

the post-injection burst activity for each unit was also normalized to its own activity prior to injection and expressed as a percentage of baseline. These data were analyzed with two-factor ANOVA (group × session) followed by Holm-Sidak post-hoc analysis.

In order to analyze responses of mPFC units to the light/tone cue presented following injections, PETHs with a bin size of 25 ms were constructed around 270 pairings with the light and tone cues. Evidence for robust changes related to these events was evaluated by calculating the firing rates for 150 ms after stimulus onset or offset and determining if these were at least 2 SDs above or below the mean firing rate during the previous 500 ms (Gulley et al., 2002).

Results

Locomotor behavior

Analysis of quadrant crossing for rats in the saline and AMPH treatment groups revealed significant main effects of group [$F(1,40) = 11.8$, $p = 0.01$] and session [$F(4,40) = 4.75$, $p < 0.01$], along with a significant group × session interaction [$F(4,40) = 5.07$, $p < 0.01$]. As shown in Fig. 5.2, repeated treatment with saline did not change subsequent behavioral responses to saline on test days 1 and 5 or on either of the challenge sessions. AMPH (1.0 mg/kg, i.p.), however, produced an increase in locomotor activity during treatment 1, and subsequent repeated exposure induced sensitized locomotor behavior that began to appear on the fifth treatment day and became statistically significant on the AMPH challenge (i.e., challenge 2). To determine whether conditioning occurred in response to repeated AMPH exposure, a saline challenge was performed on the first day after withdrawal (i.e., challenge 1). The presence of conditioning was defined as an increase in locomotor activity on challenge 1 compared

to the first injection with saline and to the activity in rats repeatedly exposed to saline. Although the number of quadrant crossings following saline challenge was greater (> 2-fold increase) than those observed after the first saline injection, this difference was not statistically significant ($p > 0.67$).

Basal mPFC activity

A total of 349 single units were recorded from the prelimbic and infralimbic regions of the mPFC during the first and fifth treatment days, as well as during challenge 1 and 2. During the 30 min they were in the open-field arena prior to injection, rats typically engaged in exploratory behaviors such as locomotion, rearing, and sniffing for the first 15 min ("active" period) and then rested with only occasional movement (e.g., sniffing, head movement, or brief body movements) for the last 15 min ("inactive" period). Table 5.1 shows the mean firing and burst rate for these periods of high and low spontaneous activity. Overall, there was little evidence of movement-related changes in activity the two treatment groups and there were no consistent changes in baseline activity across recording sessions. The one exception was the small, but significant increase in firing and burst rate that was observed in the inactive period on treatment 5 in rats from the saline-treated group. This statistically significant difference was revealed via significant main effects of session [firing rate: $F(3,696) = 5.88$, $p < 0.001$; burst rate: $F(3,649) = 3.84$, $p < 0.05$] and group [firing rate: $F(1,696) = 5.66$, $p < 0.05$; burst rate: $F(3,696) = 5.06$, $p < 0.05$] in the overall ANOVA and a significant group × baseline interaction [firing rate: $F(1,167) = 15.6$, $p < 0.001$; burst rate: $F(1,161) = 8.68$, $p < 0.01$] in follow-up analysis. Similarly, there was evidence of between-group differences in baseline mPFC activity on challenge 2 [main effect of

group; firing rate: $F(1,160) = 4.07$, $p < 0.05$; burst rate: $F(1,149) = 7.59$, $p < 0.01$], with firing and burst rate reduced in AMPH-treated rats. This result was only significant when data were collapsed across the active and inactive baselines, however, as the group × baseline interactions on challenge 2 were not significant (p values > 0.59).

mPFC activity after saline or AMPH treatment

Following the first injection with 1 mg/kg AMPH (i.e., treatment 1), the majority of mPFC neurons significantly changed firing rate compared to their own baseline and to the firing of units recorded from rats given saline (Fig. 5.4A; $\chi 2 = 16.3$, $p < 0.001$). These changes were mostly reductions in firing, with smaller populations of cells either increased or not consistently changed. The responses in saline- compared to AMPH-treated rats were also significantly different following the fifth treatment ($\chi 2 = 12.2$, $p = 0.002$). During treatment 5 compared to treatment 1 in rats given AMPH, there tended to be fewer cells that did not consistently change and more cells that increased their firing post-injection (Fig. 5.4A). However, this change in the proportion of response types was not statistically significant. In response to the first challenge, where both groups received saline injections and the AMPH group was exposed to a light/tone cue previously associated with AMPH injection, there was not a significant difference in the proportion of responses between groups. There was, however, a significant change in the proportion of responses in the AMPH groups in comparison to those observed after the first treatment ($\chi 2 = 18.4$, $p < 0.001$). Specifically, following injection there were significantly more units that did not consistently change or increased their firing and significantly fewer cells that decreased their firing. During challenge 2, when both groups were presented the light/tone cue and both received the same injection type

they had received on treatments 1 and 5, there was a significant group difference in the proportion of response types: 67% of the cells recorded from rats in the AMPH group exhibited decreases in firing following injection, whereas 59% of the cells recorded from rats in the saline group exhibited no consistent change ($\chi 2 = 22.0$, $p < 0.001$). This response pattern in the AMPH treatment group was not significantly different from that seen at treatment 1, however.

As shown in Fig. 5.4B, there were significant group differences in the magnitude of firing rate changes following injections on the treatment and challenge days. A three-way ANOVA of these data revealed significant main effects of session [$F(3,339) = 2.68$, $p < 0.05$] and response type [$F(2,339) = 66.4$, $p < 0.001$], along with significant two-way interactions [group × session: $F(3,339) = 2.89$, $p < 0.05$; group × response type: $F(2,339) = 9.25$, $p < 0.01$; session × response type: $F(6,339) = 3.60$, $p < 0.01$]. Follow-up, two-way ANOVA analyses on data from individual sessions revealed that on treatments 1 and 5, the decreases or increases in baseline that were seen in units from AMPH-treated rats were significantly different from the post-injection firing observed in units classified as not changed (Fig. 5.4B). Furthermore, the magnitude of AMPH-induced excitation was greater on treatment 5 compared to treatment 1. For units classified as increased following AMPH injection, these normalized firing rates were also significantly different from those observed in saline-treated rats. When all rats were challenged with saline following the three-day withdrawal period (i.e., Ch 1), only the units recorded from rats in the AMPH treatment group and classified as increased following injection were significantly different from those classified as not changed. Furthermore, the magnitude of this increase was significantly higher in AMPH-treated

compared to saline-treated rats. Following AMPH challenge (i.e., Ch 2), the magnitude of AMPH-induced decreases and increases in firing was similar to that seen on treatment 1, though only those classified as increased were significantly different from those classified as not changed. In the saline-group, the magnitude of the firing rate increase on challenge 2 was significantly greater than that observed during treatment 1. During this challenge session, these rats were given saline but they were also exposed to a light/tone cue for the first time.

Analysis of burst firing in the mPFC neurons recorded here revealed that AMPH tended to decrease both burst firing rate (Fig. 5.5A) and the percentage of spikes in bursts (Fig. 5.5C) compared to baseline, but this effect was only evident on the first treatment day and the AMPH challenge session. An overall three-way ANOVA of these data revealed significant main effects of session for both measures [burst rate: $F(3,649) = 6.29$, $p < 0.001$; percentage of spikes in bursts: $F(3,649) = 2.92$, $p < 0.05$]. In addition, there was a significant main effect of group for burst rate [$F(1,649) = 8.86$, $p < 0.01$] and a significant session × group interaction for percentage of spikes in bursts [$F(3,649) = 3.37$, $p < 0.05$]. To account for both within- and between-group variability in bursting, data were normalized for each cell to its pre-injection baseline. A two-way ANOVA of the normalized burst rate revealed a significant main effect of session [$F(1,273) = 3.26$, $p < 0.05$] and a significant group × session interaction [$F(3,273) = 5.16$, $p < 0.01$]. As shown in Fig. 5.5B, burst rate was significantly increased in the AMPH-treated group at challenge 1 when compared to the saline group and to the burst rate in cells recorded form the AMPH group at the first treatment. On challenge 2, the treatment groups were also significantly different from each other, with the saline group

having a higher burst rate. As shown in Fig. 5.5D, the percentage of spikes in bursts was significantly reduced in the AMPH- compared to the saline-treated group during treatment 5 and challenge 2. At challenge 1, there was a significant increase in the percentage of spikes in bursts in units recorded from the AMPH-treated group compared to those treated with saline and to units recorded from the AMPH group after the first injection with AMPH.

In order to assess the potential for modulations in mPFC firing rate that were specific to the cues presented to rats in the AMPH group following injections with AMPH on treatments 1 and 5 and challenge 2, or following saline on challenge 1, PETHs were constructed with cue onset or offset as the reference event. We found no evidence of statistically significant, cue-related modulations in firing during any of the treatment or challenge sessions (data not shown). When this same analysis was done for saline-treated rats on challenge 2, we also did not find any units that exhibited significant cue-related changes in firing (data not shown).

Discussion

The goal of this study was to investigate AMPH-induced adaptations in the function of mPFC neurons that, based on results from previous anatomical and *in vitro* electrophysiological studies, are expected to emerge along with the behavioral adaptations that typically result from repeated AMPH exposure (i.e., behavioral sensitization). This was accomplished by utilizing chronically implanted microwire electrodes to record the activity of mPFC neurons during a baseline period and after injections of either saline or AMPH in rats there were allowed to behave in an open-field

arena. We found that during baseline recording (i.e., pre-injection), neurons in the mPFC generally did not modulate their spiking activity during periods of spontaneous movement relative to periods of inactivity. Furthermore, in rats given AMPH repeatedly, there was no consistent, statistically significant change in baseline firing or burst rate across treatments or AMPH challenges. Thus, repeated AMPH exposure did not produce a significant "hypofrontal" state as might be predicted from studies in humans with a long history of cocaine abuse (Goldstein and Volkow, 2002) and those in rodents showing that PFC-mediated glutamate release in the NAc is reduced in cocaine-sensitized rats (Pierce et al., 1996; Hotsenpiller et al., 2001; McFarland et al., 2003). Reduced baseline spiking activity in the mPFC has also been reported in rats with a relatively long history of cocaine self-administration experience (Sun and Rebec, 2006). Consistent with the present results, however, is the finding that repeated exposure to 0.5 or 2.0 mg/kg AMPH had no significant effect on baseline mPFC activity recorded from behaving rats (Homayoun and Moghaddam, 2006). Thus, the development of drug-induced hypofrontality in the mPFC may be influenced by the duration of drug exposure, whether the drug is self-administered or given non-contingently, and also by the type of drug.

Following its first injection, 1.0 mg/kg AMPH had primarily inhibitory effects on both firing rate and bursting in the mPFC. Mostly inhibitory responses have been described previously in anesthetized rats given a single injection of 1 or 2 mg/kg AMPH (Mora et al., 1976) and in brain slices where local application of AMPH leads to a depression of excitatory field potentials in layer V neurons that are evoked by layer II/III stimulation (Mair and Kauer, 2007). In recordings from awake, behaving rats, inhibitory

responses also predominated following a single dose of 2 mg/kg AMPH, whereas excitatory responses are more common following acute exposure to 0.5 mg/kg AMPH (Homayoun and Moghaddam, 2006). When taken with the latter study, the present results suggest the primary response in the mPFC to lower AMPH doses is opposite to that seen with higher doses. This may be relevant for the therapeutic effects of AMPH in disorders such as attention deficit hyperactivity (ADHD) where the effective dose range is typically 0.2-0.5 mg/kg (Solanto, 2000).

By the fifth injection of AMPH, when locomotor sensitization began to emerge, the pattern of mPFC unit responses to the drug was generally similar to that observed following the first injection – AMPH-induced inhibitions in firing rate predominated. However, the magnitude of excitatory responses, which represented 24% of the recorded neurons, was significantly increased from ~ 267% of baseline to ~474% of baseline on treatment 1 and 5, respectively. Following AMPH challenge, these drug-induced excitations were also of a greater magnitude (~350% of baseline) compared to treatment 1. Importantly, this occurred when locomotor sensitization to AMPH was clearly evident. These changes in the magnitude of excitation were also associated with decreases in the rate of bursting during AMPH challenge and decreases in the number of spikes in bursts during treatment 5 and the AMPH challenge. It may be the case, therefore, that in behaving animals, the population of neurons excited by AMPH is more vulnerable to the plasticity induced by repeated AMPH exposure compared to the population inhibited by the drug. This hypothesis requires further study, particularly using techniques that allow for the simultaneous analysis of behavior and neuronal function.

Relative to units recorded from saline-treated rats, we observed increased burst activity associated with increases in locomotion following saline challenge in the AMPH-treated group. This may be related to an increased ability to process drug-paired information (Childress et al., 1999; Rebec and Sun, 2005; Sun and Rebec, 2006), as the PFC is well known to be involved in learning-related plasticity in rats (Mulder et al., 2003; Schoenbaum et al., 2003; Bouret and Sara, 2004). A specific role for associative conditioning in AMPH-induced adaptations in the mPFC was also highlighted in a recent study showing that drug-induced changes in dendritic morphology in the mPFC were only observed in those rats given systemic injections of AMPH and not those given local AMPH infusions into the ventral tegmental area (Singer et al., 2009). Both of these routes of AMPH exposure induce locomotor and neurochemical sensitization in the NAc, but only systemic exposure produces conditioned responding (Stewart and Vezina, 1991; Vezina, 1996). An alternative hypothesis is that the increased firing rate and burst activity we observed could have resulted in response to the novel situation presented by the first pairing of cues (tone and light) with saline rather than AMPH. While this explanation is plausible, it is noteworthy that saline-treated rats did not show a statistically significant increase in burst activity when they experienced this novel situation for the first time (i.e., challenge 2 where saline injection was paired to the tone and light cues). Thus, the changes in mPFC activity observed following saline challenge are more likely related to the conditioned behavior observed and may represent a change in responsiveness to the overall environmental context. The lack of temporal specificity we observed in our PETH analysis of cue-specific modulations in mPFC activity may also be a reflection of this more global response to the drug-

associated context.

The PFC is part of a widely distributed neuronal network that has extensive reciprocal connections with both cortical and subcortical regions (Kolb, 1984). Thus, it is positioned to play a critical role in organizing behavior through functional regulation of numerous subcortical structures. The pyramidal cells in the mPFC, which are more than likely the source of the unit responses we recorded, are known to project densely to limbic structures such as the NAc, hippocampus, and amygdala (Sesack et al., 1989; Vertes, 2002; Heidbreder and Groenewegen, 2003). Moreover, the prelimbic and infralimbic areas receive the densest innervation of VTA dopamine efferents relative to dorsal regions of the mPFC (Lindvall et al., 1978). Thus, it was somewhat unexpected to find that neuroadaptations in the response to AMPH in the mPFC were not as apparent as those observed for AMPH-induced locomotor behavior. It is likely that AMPH-induced adaptations in the mPFC are dependent on the exposure dose, method of drug administration, and duration of withdrawal before challenge. Unfortunately, no other published studies have systematically described mPFC plasticity concurrently with AMPH-induced adaptations in the behavioral response. It has been reported, however, that higher doses of repeated AMPH or cocaine treatment, along with longer withdrawal time periods, altered the electrophysiological properties of mPFC neurons *in vitro* (Peterson et al, 2006), in anesthetized rats *in vivo* (Onn and Grace, 2000; Peterson et al., 2000; Trantham et al., 2002), and in awake rats (Homayoun and Moghaddam, 2006; Sun and Rebec, 2006). In the only other published report of mPFC recording in behaving rats, 2 mg/kg AMPH given once per day for 5 days was reported to shift mPFC responses to AMPH such that a greater number of AMPH-induced inhibitions

were observed when a challenge injection of AMPH was given 10 days after the fifth drug treatment. A second AMPH challenge given 14 days after the first revealed that this response to AMPH persisted (Homayoun and Moghaddam, 2006). In this study, however, locomotor activity data were not presented, so it is unclear if the observed plasticity in the mPFC was associated with adaptations in the behavioral response to AMPH at both of the challenges. Because the recordings in this study were obtained while rats were in their home cages, it is possible that the inconsistencies with our results are related to the absence of AMPH-induced locomotor sensitization. The importance of environmental context for the development and expression of psychostimulant sensitization has been well documented (Badiani and Robinson, 2004), and single-unit recordings from the mPFC have highlighted its sensitivity to context-discrimination and sensorimotor information (Gemmell et al., 2002; Baeg et al., 2003; Euston and McNaughton, 2006; Cowen and McNaughton, 2007). Thus, it is conceivable that different neuroadaptations can be induced by repeated AMPH exposure and that they are influenced by the behavioral state of the animal.

In summary, we found that repeated exposure to AMPH, using a treatment schedule that resulted in locomotor sensitization to AMPH, leads to selective and relatively modest changes in mPFC function. In particular, AMPH-induced increases in activity were enhanced following five days of repeated treatment and persisted, but to a lesser extent, during an AMPH challenge given following a 3-day withdrawal period. AMPH-induced decreases in mPFC activity, which were the more frequently encountered type of response, were not significantly changed over the course of treatment or following challenge injections. When taken with other studies in behaving

rats where repeated cocaine (Sun and Rebec, 2006) or AMPH (Homayoun and Moghaddam, 2006) were shown to produce more robust and longer lasting changes in baseline mPFC function and in the response of mPFC neurons to drug challenges, our findings suggest that AMPH-induced plasticity in the mPFC is highly dependent on treatment factors such as dose, duration of withdrawal, and the treatment environment. Furthermore, they highlight the importance of measuring the neuronal and behavioral consequences of repeated drug exposure concurrently. By doing so, it is possible to more directly correlate neural adaptations with the expression (or absence) of behavioral adaptations. This is particularly important in regards to the mPFC because it has been demonstrated that sensitizing regimens of AMPH treatment have long lasting effects on drug-induced locomotor behavior but do not significantly alter mPFC-sensitive behavioral tasks such as delay-discounting (Stanis et al., 2008b), delayed non-matching to position (Featherstone et al., 2008), or delayed spatial alternation (Stefani and Moghaddam, 2002).

CHAPTER 6: GENERAL DISCUSSION

Impulsivity and cognitive dysfunction are typically reported and possibly overlapping traits for a number of psychiatric disorders, including addiction (Dolan et al., 2008; Ersche et al., 2012). While we hypothesize that drug-induced changes in brain function are a major contributing factor to the behaviors that characterize addiction, it is not necessarily clear whether co-morbid personality traits like impulsivity and cognitive dysfunction predate the onset of drug use or occur as a consequence of chronic use. It is known, however, that the progression from initial use to dependence involves synaptic plasticity in a number of brain pathways, with glutamate and dopamine systems being crucial for the ensuing plasticity (Seamans and Yang, 2004; Wolf et al., 2004; Kalivas et al., 2008). Increasing evidence points to the mPFC as a key region of regulating the manifestation of adaptations in response to chronic drug exposure (Li et al., 1999; Tzschentke and Schmidt, 1999; Bjijou et al., 2002; Morshedi et al., 2009; Fanous et al., 2011). The purpose of this thesis was to examine the long-lasting effects of repeated AMPH exposure on cortical function and cognitive behavior.

The experiments presented here first evolved from an initial study looking at the relationship between the locomotor response (i.e., the ability to induce sensitization) and mPFC firing activity in response to repeated AMPH treatment. This study was based on the current theories that suggest neuroadaptations in the PFC are responsible for driving behavioral changes associated with chronic psychostimulant exposure (Goldstein and Volkow, 2002; Koob and Volkow, 2010; Kalivas and Volkow, 2011). We implanted electrodes into the mPFC and recorded neuronal activity while rats were actively behaving in an open-field chamber before and after injections of AMPH (1.0

mg/kg). We found that repeated AMPH led to very selective and modest changes in mPFC function when behavioral sensitization was present. More specifically, an acute injection had primarily inhibitory effects on both firing rate activity and bursting patterns. These findings agree with recordings from anesthetized rats and in vitro studies (Mora et al., 1976; Mair and Kauer, 2007). After five injections, when locomotor sensitization emerged, the magnitude of excitatory responses increased. These changes were less persistent following AMPH challenge, which occurred after a three-day washout period (to ensure that the effects were due to long-lasting alterations rather than metabolic side effects). The magnitude of excitation was also coupled with reduced rate and number of spikes within bursting. Interestingly, during a cue challenge (i.e., saline injected in the presence of drug-associated cues), the AMPH-treated rats showed an increase in bursting activity. Taken together, our data suggest that particular populations of neurons are more vulnerable to AMPH-induced plasticity. This vulnerability can be interpreted in light of recent findings that suggest transient changes in specific PFC projection neurons (Hedou et al., 2002; Fanous et al., 2011). In addition, several studies show that sensitizing regimens of AMPH does not necessarily lead to long-lasting changes in behavioral tasks that are sensitive to changes in PFC function (Stefani and Moghaddam, 2002; Featherstone et al., 2008). Focusing on plasticity within specific pathways will be key in future studies.

We then wanted to examine the relationship between behavioral sensitization and impulsive choice behavior in the same animals after chronic drug treatment. Rats were first repeatedly treated with AMPH and then trained in a delay-discounting task, which is sensitive to the rats' inability to tolerate longer delays for a more advantageous

food reward. We found that chronic exposure to AMPH did not cause rats to become more impulsive (saline- and AMPH-treated rats were equally impulsive), but we observed long-lasting changes in AMPH-induced locomotor activity. These results were somewhat surprising because they suggested that the neuroadaptations, which are known to be associated with intermittent exposure and behavioral sensitization (Robinson and Berridge, 2000), did not cause enduring deficits in the aspects of cognition that the delay-discounting task requires. While a number of studies suggest that impulsivity is modulated by DA (van Gaalen et al., 2006; Dalley et al., 2011; Economidou et al., 2012), the exact mechanisms are not yet clear and could involve both cortical and subcortical changes. Furthermore, there are a number of behavioral tasks that measure aspects of impulsive behavior, so it is entirely possible that DA modulation produces more specific effects in other impulsive choice tasks, as reported in the literature (Peterson et al., 2003; Eagle et al, 2007; Navarra et al., 2008). It is notable, however, that studies using similar AMPH treatment schedules as the one used here have reported impairments in both subsequent structural plasticity and memory tasks (Bisagno et al., 2003; Kolb et al., 2003; Briand et al., 2005; Castner et al., 2005; Belcher et al., 2006; Fletcher et al., 2007). Our data thus support the possibility that impulsivity might be a pre-existing trait, and this notion inspired our next experiment.

Not all drug users become addicted. It is estimated that only 10-15% of initial users ultimately become dependent (Gawin, 1991). Based on statistics linking an initial responsiveness of a psychostimulant to the long-term rate of use and abuse, it is intriguing to think that one might be able to predict long-term deficits based on one single exposure. We, and others (Sabeti et al., 2002; Sabeti et al., 2003; Gulley et al.,

2003), have used the initial drug response to classify outbred, male, Sprague-Dawley rats based on the median split of locomotor activity for the first 30 minutes following an acute injection of a low dose of cocaine (10 mg/kg). As reported in Chapter 3, rats were classified into LCRs and HCRs and then trained on a delay- discounting task. Again, this task is extremely sensitive to rats' inability to tolerate long delays for larger food reinforcement. To our surprise, we found that HCRs were more impulsive than LCRs. The results were somewhat unexpected because previous studies have shown that LCRs are more sensitive to the discriminative stimulus properties of cocaine (Klein and Gulley, 2009), show conditioned place preference for cocaine (Allen et al., 2007), and self-administer cocaine more readily than HCRs (Mandt et al., 2008). Taken together, those results suggest that LCRs are more vulnerable to the rewarding and reinforcing properties of cocaine. Considering that DA is primarily important for the acute response, as the first injection of cocaine inhibits DAT-mediated clearance of DA and increases extracellular DA in the nucleus accumbens and dorsal striatum to a greater extent in HCRs than LCRs (Sabeti et al., 2002; Nelson et al., 2009), it is logical that HCRs behaved more impulsively. Previous studies have shown that the depletion of DA or infusion of DA receptor antagonists in the ventral striatum profoundly reduced impulsivity (Cole and Robbins, 1989; Pattij et al., 2007; Pezze et al., 2007), which is in agreement with our findings in LCRs. Hence, it is not surprising that LCRs and HCRs differed in impulsive behavior.

A final set of experiments were conducted to determine: (1) if repeated AMPH exposure would produce more significant impairments in a task that, relative to delay-discounting, has been shown consistently to be highly sensitive to changes in cortical

function (Fuster and Alexander, 1971; Kolb, 1990; Goldman-Rakic, 1995; Dias and Aggleton, 2000; Brown and Bowman, 2002); (2) if a model of repeated AMPH exposure that more closely approximates human patterns of drug intake would produce more significant cognitive impairments than are seen with an intermittent exposure model; (3) if the age of exposure contributes to AMPH's ability to produce cognitive deficits, considering that adolescence is an important period of normal developmental flux; and (4) if subsequent cognitive function can be predicted based on an animal's initial sensitivity to a psychostimulant during both adolescence and adulthood.

We found an inverse relationship between the robustness of a sensitized locomotor response and ensuing cognitive deficits after repeated AMPH exposure. That is, while adolescent rats were less sensitive to the stereotypy-inducing effects of both an intermittent and escalation-binge method, they were more vulnerable to exposure-induced learning and memory impairments. Current evidence suggests that the nature and timing of normal developmental changes interacts with the changes occurring in response to drug exposure (see Brenhouse and Andersen, 2011). A long history of experience-dependent changes in the developing brain exists, including experiences with psychoactive drugs (Diaz Heijtz et al., 2003; Robinson and Kolb, 2004), sensory and motor enrichments (Rosenzweig et al., 1962; Greenough and Chang, 1988; Kolb et al., 2003; Mychasiuk et al., 2012), parental-infant and peer relationships (Weaver et al., 2004; Bell et al., 2010), and stress (Andersen and Teicher, 2008; Muhammad and Kolb, 2012).

Optimal levels of DA are critical to proper function of the PFC (Arnsten and Pliszka, 2011). One plausible mechanism then is that the normal developmental

process of overproduction and regressive elimination that fine tunes the brain for efficiency is dramatically altered in response to drug exposure. Thus, overproduction and pruning of D1 receptors on glutamatergic output neurons during development (at PND40) could be responsible for the enduring changes (Brenhouse et al., 2008). It is known that cortical development lags in children with ADHD (Shaw et al., 2007), but is accelerated in schizophrenia (Rapoport et al., 1999). Nonetheless, a shift in the pruning response could lead to long-lasting changes, and such effects would agree with the "equal, but opposite" model of enduring drug action (Andersen, 2003, 2005). Briefly, this model states that whatever long-term effects are produced during adulthood, the opposite effect will be true following drug administration during adolescence. Thus, chronic exposure to a lower dose during adolescence has the potential to exacerbate symptoms later in adulthood. In agreement then with the working hypothesis, adult-exposed rats in the escalation-binge treatment group showed delay-dependent deficits that persisted through criterion, overtraining, and task reversal. Because adolescent-exposed rats showed improvements in memory function over time, unlike the adult-exposed rats, we conclude that adults are more prone to the neurotoxic effects of high-dose AMPH treatment. Support of this notion comes from rats exposed to escalating dose-multiple binge exposure of methamphetamine (Kuczenski et al., 2007).

Interestingly, the delay dependent deficits in adolescent- and adult-exposed rats were predicted based on their initial sensitivity to a single injection of cocaine. In terms of locomotor responses after repeated exposure, however, the differences between LCRs and HCRs disappeared in parallel with the development of behavioral sensitization. Because there are no pharmacokinetic differences after the acute drug

response (Gulley et al., 2003), it is unlikely that metabolic differences are responsible for the long-term changes. To our knowledge, there are no reports of age-dependent differences in AMPH metabolism with repeated treatment regimens (see Cho et al., 2001). It should be noted that rats metabolize stimulants rapidly (half-life of 70 minutes versus 12 hours in humans), and consequently, drug pharmacokinetics need to be considered more so when interpreting data across species. Therefore, one proposed mechanism to explain the memory deficits is an alteration of NMDA and AMPA receptors. There are known differences between LCRs and HCRs in levels of these glutamate receptors following both acute and repeated psychostimulant exposure (Yamamoto and Zahniser, 2012). Taken together with the documented role of NMDA and AMPA receptors in the role of working memory, (Rotaru et al., 2011; Arnsten et al., 2012; Wang et al., 2013; Zhang et al., 2013), the LCR/HCR model becomes very attractive to further exploration.

In summary, our data suggest that repeated AMPH has long-lasting consequences on learning and memory processes that persist well after drug exposure has ceased. These deficits appeared to be closely associated with initial differences in cocaine-induced locomotor activity. Importantly, these deficits were dependent on both the age and method of drug exposure. While psychostimulants have a well-documented ability to alter or reduce plasticity, higher escalating-binge doses might have the potential to change brain structure and function permanently. With that in mind, perhaps clinicians should think twice before prescribing a young child a repetitive dosing regimen of AMPH, especially if a therapeutic advantage is unclear. Without a doubt, more research is warranted to determine the individual persistence of damage

caused by both intermittent and escalating-binge exposures at vulnerable times of development and maturity. Nonetheless, it is clear that a systems research approach will be necessary to examine the long-lasting course of dysfunction that is observed in adulthood.

CHAPTER 7: FIGURES AND TABLES

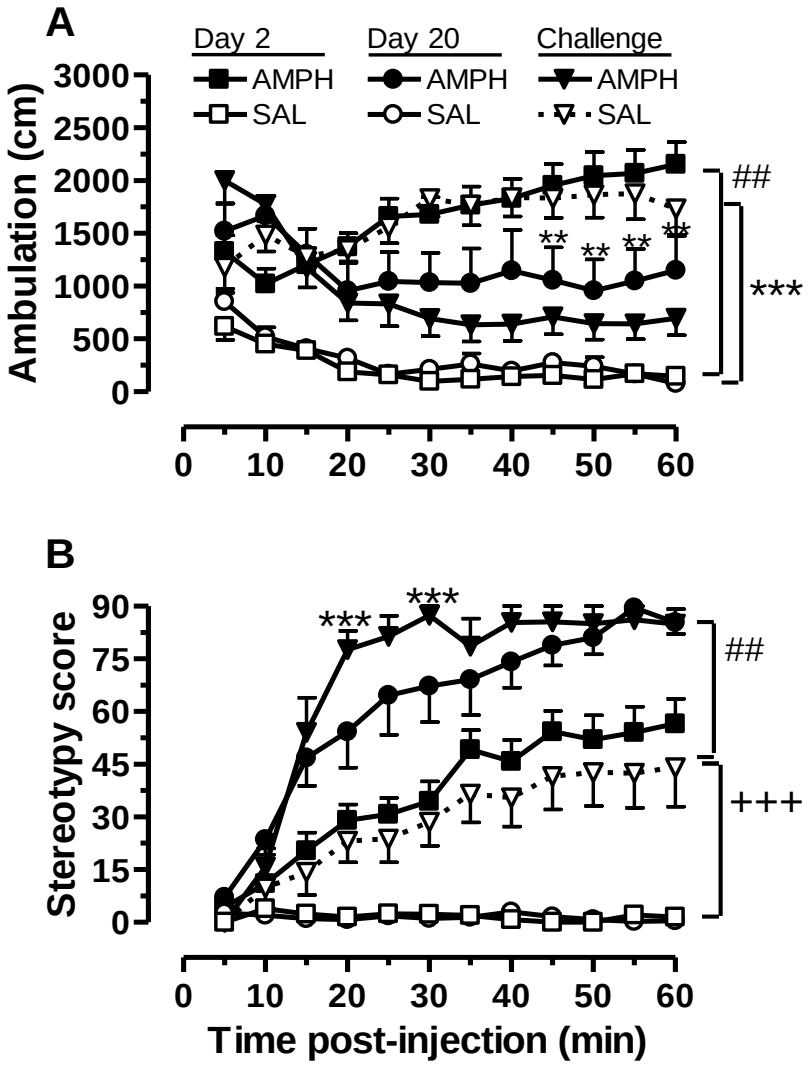


Fig. 2.1. Locomotor activity (ambulatory distance; **A**) or stereotypy rating **(B)** after acute AMPH or saline (Day 2), repeated AMPH or saline (Day 20), and the AMPH challenge given at the conclusion of the operant behavior phase of the study. AMPH was always given at a dose of 3 mg/kg (i.p.). Shown in the AMPH group are the data from 12 of 15 subjects tested because technical problems resulted in the loss of data from three subjects. **p<.01, Day 2 compared to Day 20 within the AMPH group; ##p<.01, between group comparison on Day 2; +++ p<.001, challenge compared to Day 2 and Day 20 within the SAL group; *** p<.001, challenge compared to Day 20 within the AMPH group; ###p<.001, between group comparison on challenge

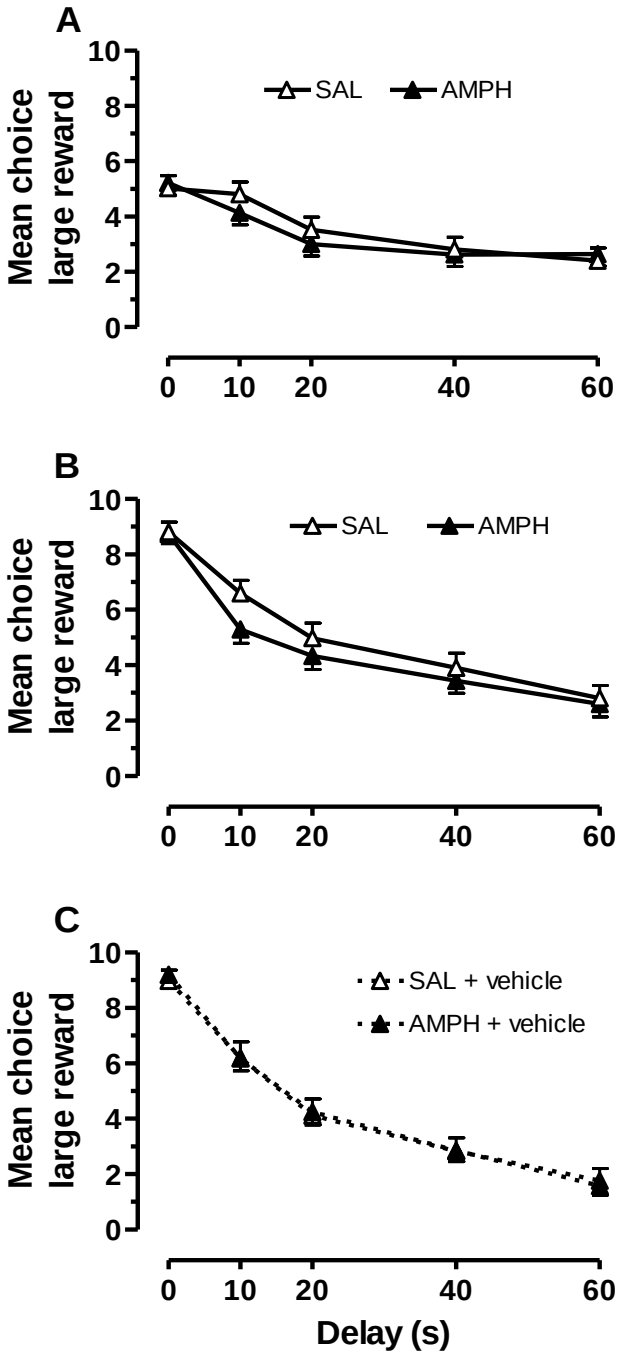


Fig. 2.2. Choice behavior for rats in the saline (SAL; n=14) and AMPH (n=15) pretreatment groups for early acquisition (sessions 1-3; **A**), training midpoint (sessions 18-20; **B**), and at stable baseline (vehicle sessions 41, 44, and 47; **C**).

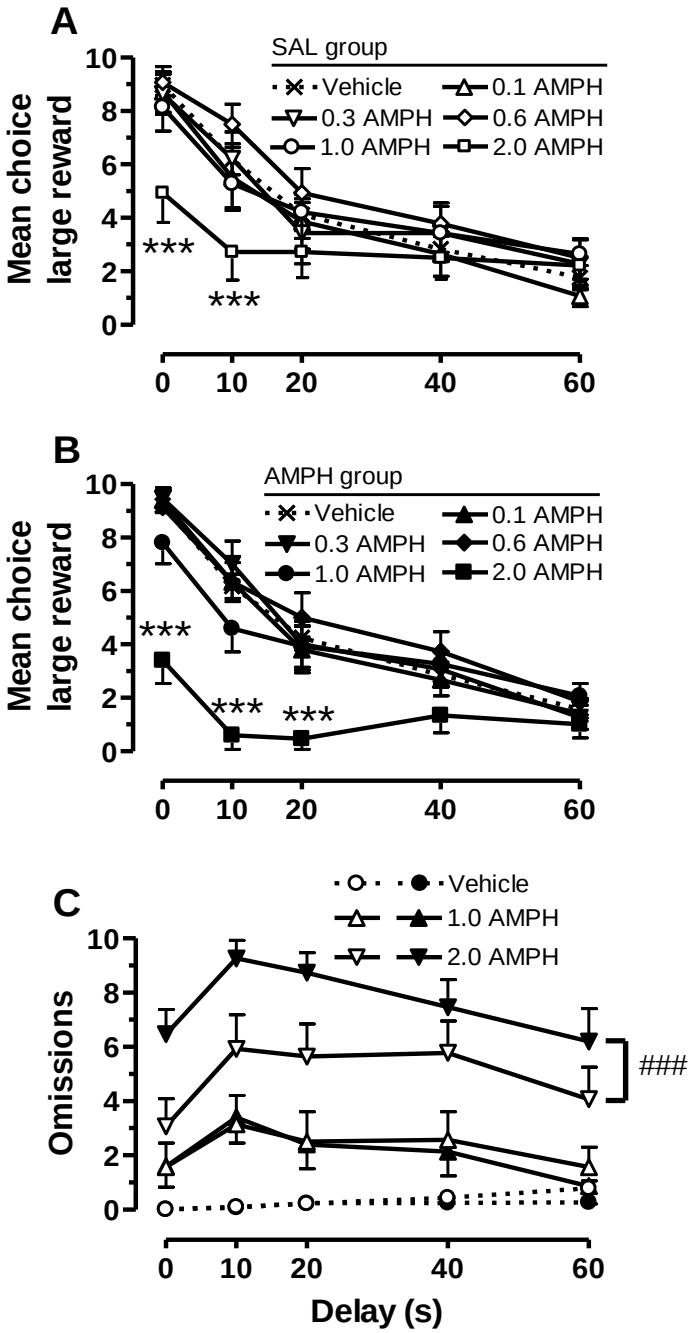


Fig. 2.3. The effects of AMPH (0.1-2.0 mg/kg) on delay-discounting behavior in rats pre-treated with saline or AMPH. Shown are the mean choice of the large reward for the saline (SAL; **A**) and AMPH (**B**) pretreated groups across delays. (**C**) Shown are the number of omissions as a function of delay for the SAL (open symbols) and AMPH (filled symbols) pre-treatment groups. For enhanced presentation clarity, only the highest doses of AMPH are included. *** p<.001, compared to vehicle at the same delays; ###p<.001, collapsed across delay

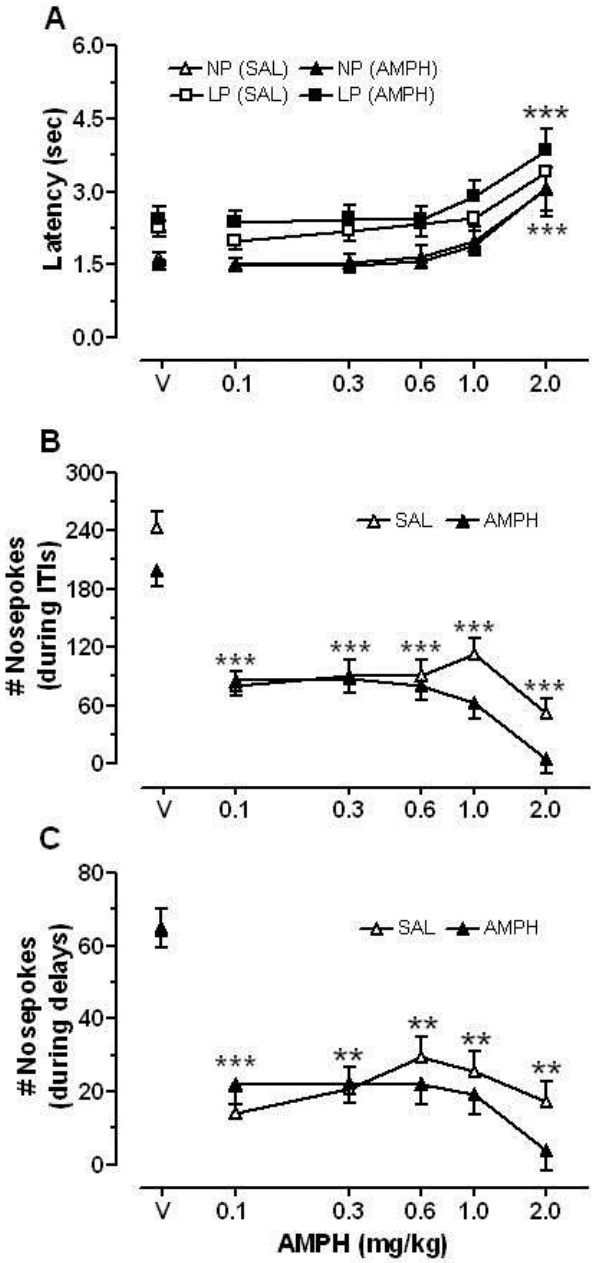


Fig. 2.4. Dose-response curves showing the effects of AMPH (0.1-2.0 mg/kg) on (**A**) latency to nosepoke for the initiation of a trial (NP) and to choose a lever (LP), and (**B**) the total number of nosepoke responses during ITIs (**B**) and delays (**C**). **p<.01 and *** p<.001, compared to vehicle (V)

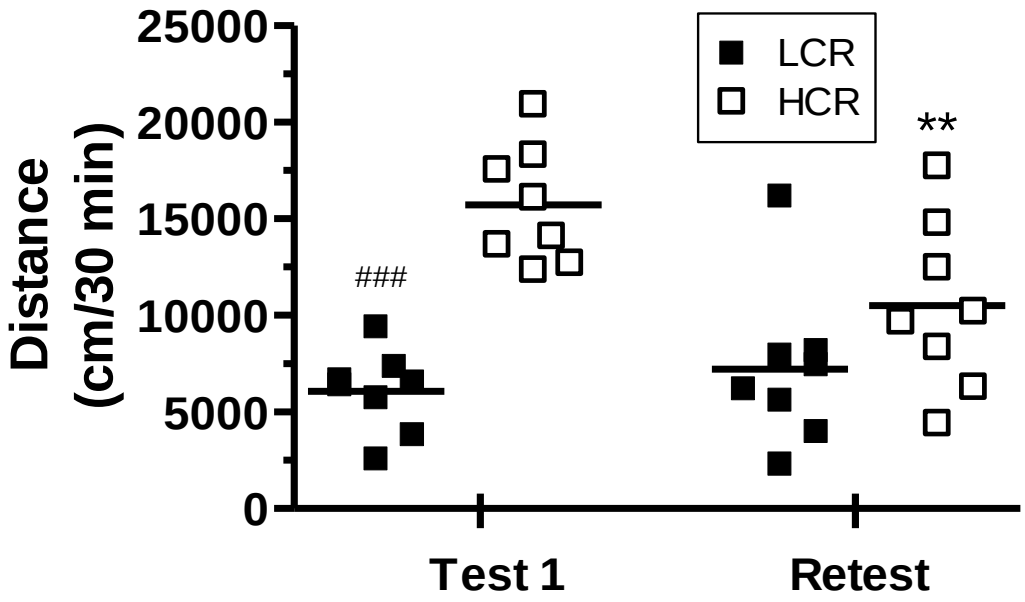


Fig. 3.1. Locomotor activity (distance, in cm) after the first (Test 1) and second (Retest) injection with 10 mg/kg cocaine. The retest occurred following the conclusion of the operant behavior phase of the study, with ~ 3 months separating the two tests. Shown is a scatter plot of the cumulative locomotor activity (mean shown as a horizontal line) for the 30-min following i.p. injection. The response during Test 1 was used to classify rats as LCRs or HCRs (n = 8/group). ### $p < 0.001$, compared to HCRs at Test1; ** $p < 0.01$, Test 1 compared to Retest within HCRs

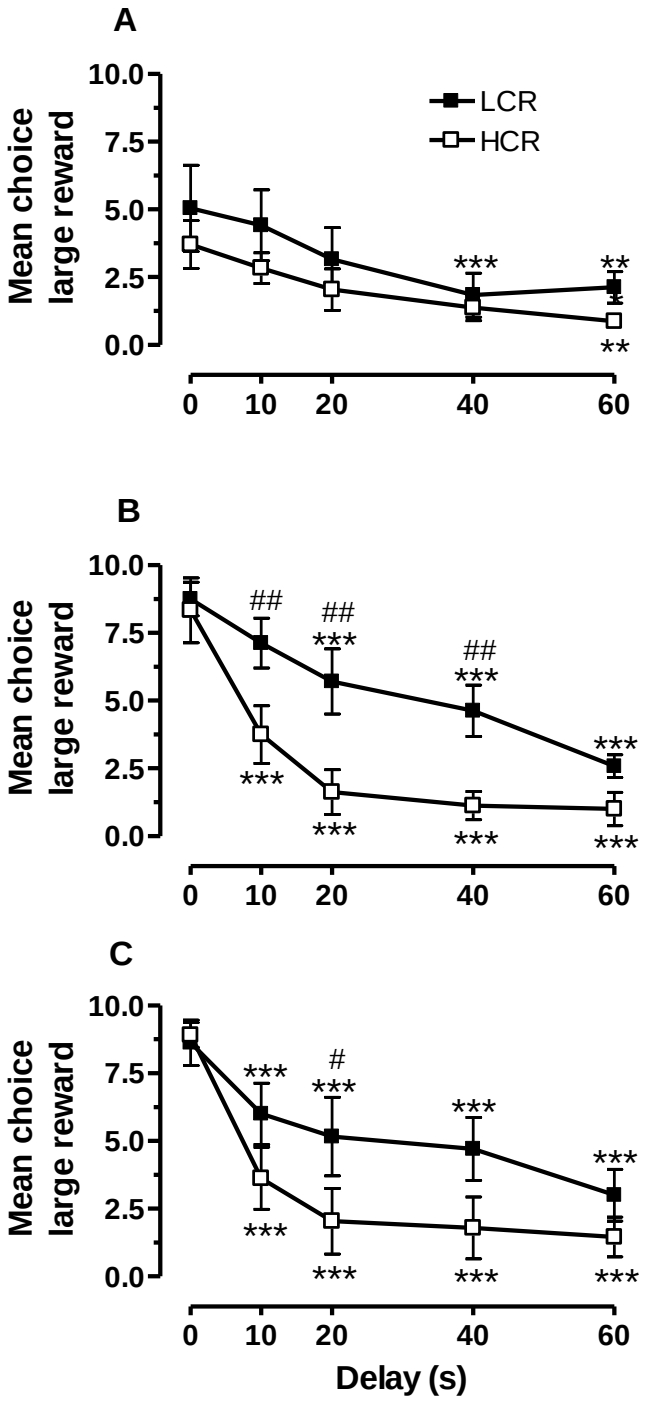


Fig. 3.2. Choice behavior during early acquisition (sessions 1-3; **A**), training midpoint (sessions 15-17; **B**), and at stable baseline (vehicle sessions 35, 37, and 39; **C**) for rats classified as LCRs or HCRs (n = 8/group). Shown is the mean choice of the large reward (± SEM) at each delay during the three sessions that were within these training time periods. ** $p < 0.01$ and *** $p < 0.001$, compared to 0 s; # $p < 0.05$ and ## $p < 0.01$, compared to HCRs at the indicated time points

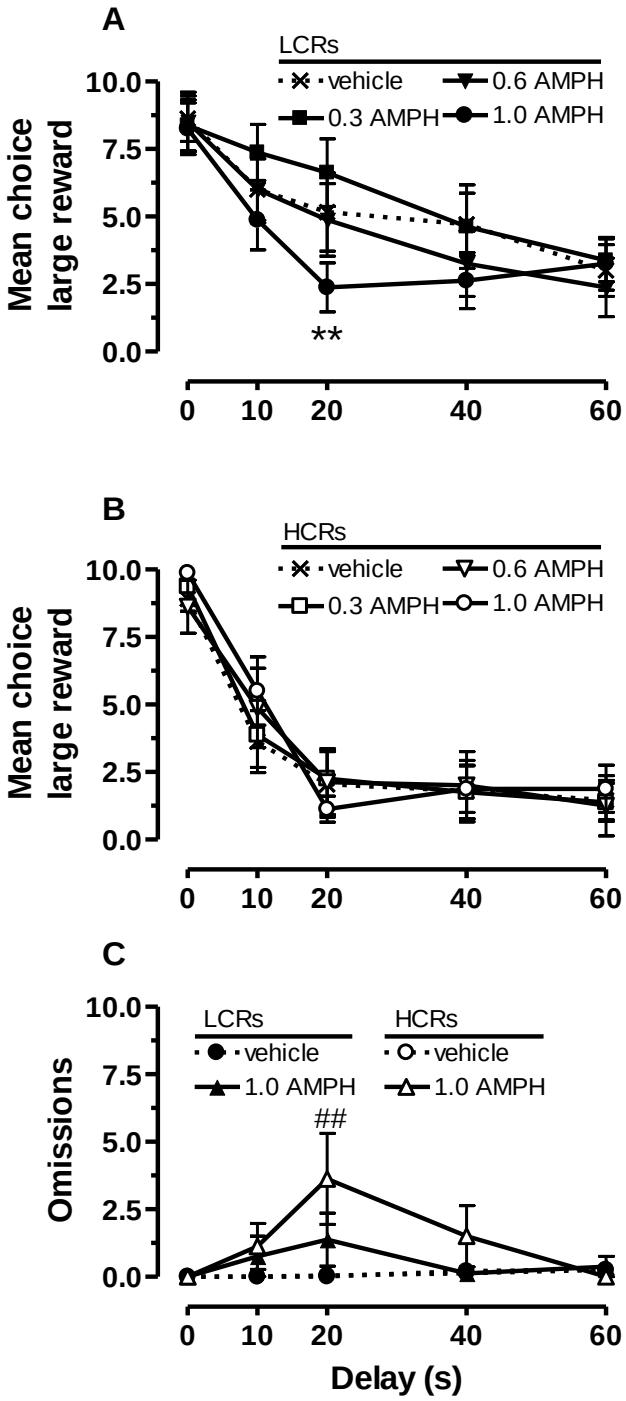


Fig. 3.3. The effects of AMPH (0.3-1.0 mg/kg) on delay-discounting behavior in LCRs and HCRs (n = 8/group). Shown in **A** and **B** is the mean choice of the large reward (± SEM) across delays for LCRs (**A**) and HCRs (**B**). Vehicle injection data are the same as that shown in Fig. 2C. The mean number of trial omissions (± SEM) as a function of delay is shown in **C**. Only the 1.0 mg/kg dose of AMPH is shown because there were no significant increases in omissions at 0.3 or 0.6 mg/kg. ** $p < 0.01$, compared to vehicle at the 20-s delay; ## $p < 0.01$, compared to vehicle at the 20-s delay for HCRs

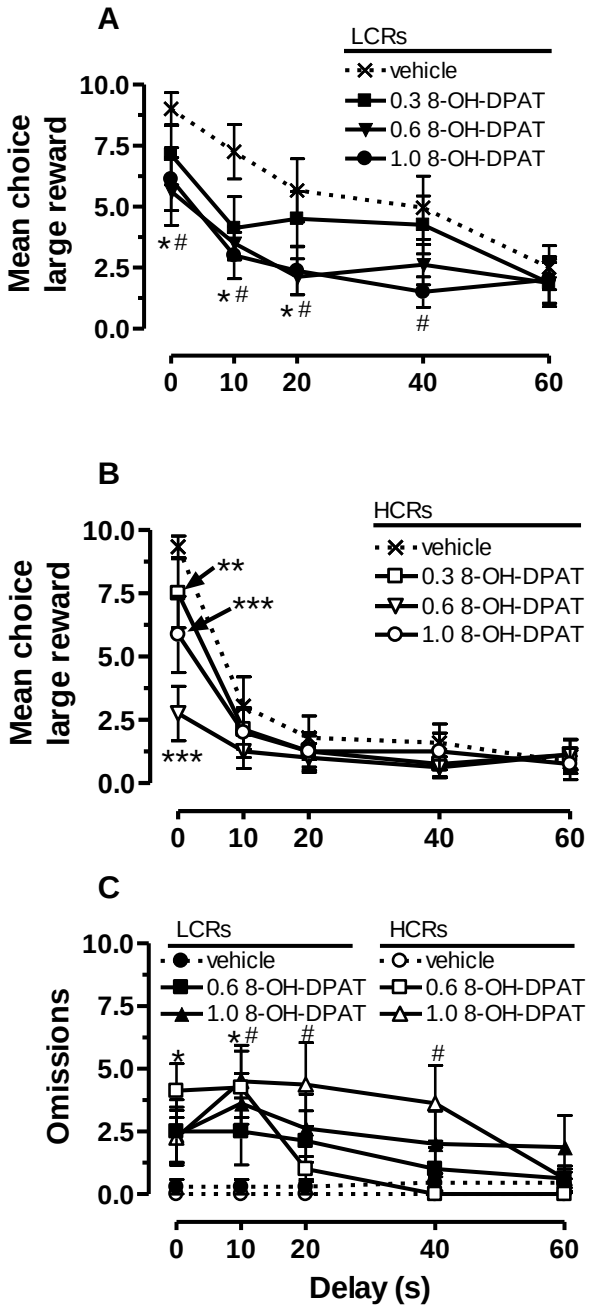


Fig. 3.4. The effects of 8-OH-DPAT (0.3-1.0 mg/kg) on delay-discounting behavior in LCRs or HCRs (n = 8/group). Shown in **A** and **B** is the mean choice of the large reward (± SEM) across delays for LCRs (**A**) and HCRs (**B**). The mean number of trial omissions (± SEM) as a function of delay is shown in **C**. The 0.3 mg/kg dose is not shown because it did not significantly increase trial omissions at any of the delays. For A: * $p < 0.05$, comparison between 0.6 mg/kg and vehicle at the indicated delay; # $p < 0.05$, comparison between 1.0 mg/kg and vehicle at the indicated delay; For B: **p<.01 and *** p<.001, compared to vehicle at the 0-s delay. For C: * $p < 0.05$, comparison between 0.6 mg/kg and vehicle at the indicated delay for HCRs; # $p < 0.05$, comparison between 1.0 mg/kg and vehicle at the indicated delay for HCRs

	AMPH (mg/kg)				8-OH-DPAT (mg/kg)			
	Vehicle	0.3	0.6	1.0	Vehicle	0.3	0.6	1.0
LCR								
Nosepoke	1.82 (0.25)	1.34 (0.14)***	1.72 (0.19)	1.77 (0.18)	1.55 (0.17)	1.87 (0.18)	2.42 (0.53)**	2.77 (0.48)***
Lever press	2.82 (0.26)	2.11 (0.23)**	2.71 (0.27)	2.68 (0.28)	2.50 (0.25)	2.92 (0.49)	3.90 (0.73)	4.64 (0.89)
HCR								
Nosepoke	1.57 (0.14)	1.30 (0.10)	1.38 (0.10)	1.41 (0.11)	1.55 (0.10)	1.81 (0.12)	2.20 (0.19)	2.26 (0.24)
Lever press	2.33 (0.33)	2.07 (0.26)	2.29 (0.31)	2.28 (0.35)	2.47 (0.25)	3.34 (0.33)	5.25 (0.87)**	4.28 (0.82)

Table 3.1. Mean latencies (s) to nosepoke for the initiation of a trial and to choose a lever, following injection of vehicle, AMPH or 8-OH- DPAT. SEM is given in parentheses. ** *p<0.01* and *** *p<0.001,* compared to vehicle

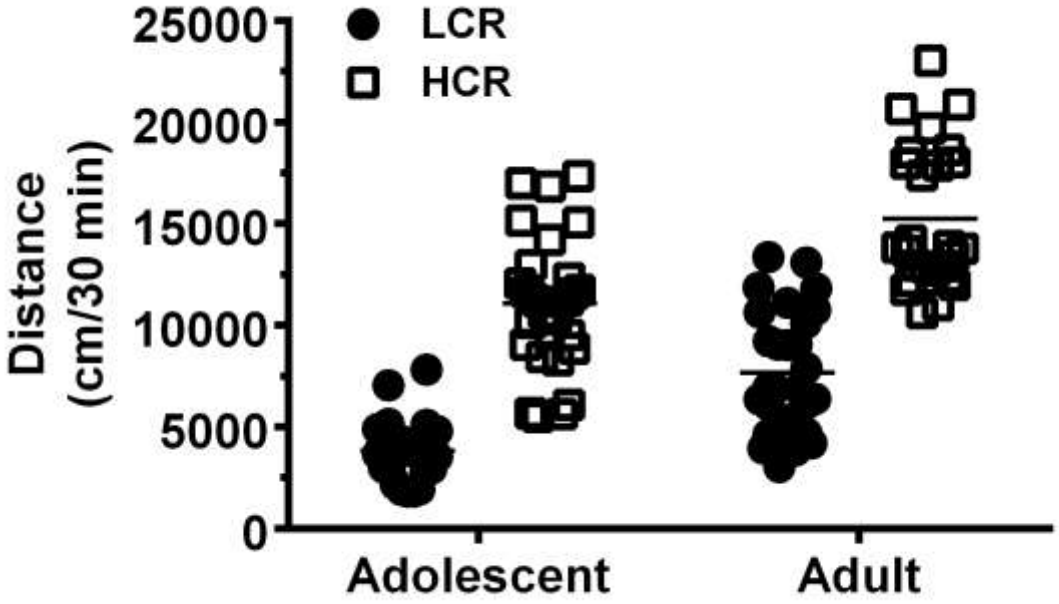


Fig. 4.1. Locomotor activity (distance, in cm) after the initial injection with 10 mg/kg cocaine in adolescent rats (PND35) and adult (PND90) rats. This response was used to classify rats as LCRs or HCRs.

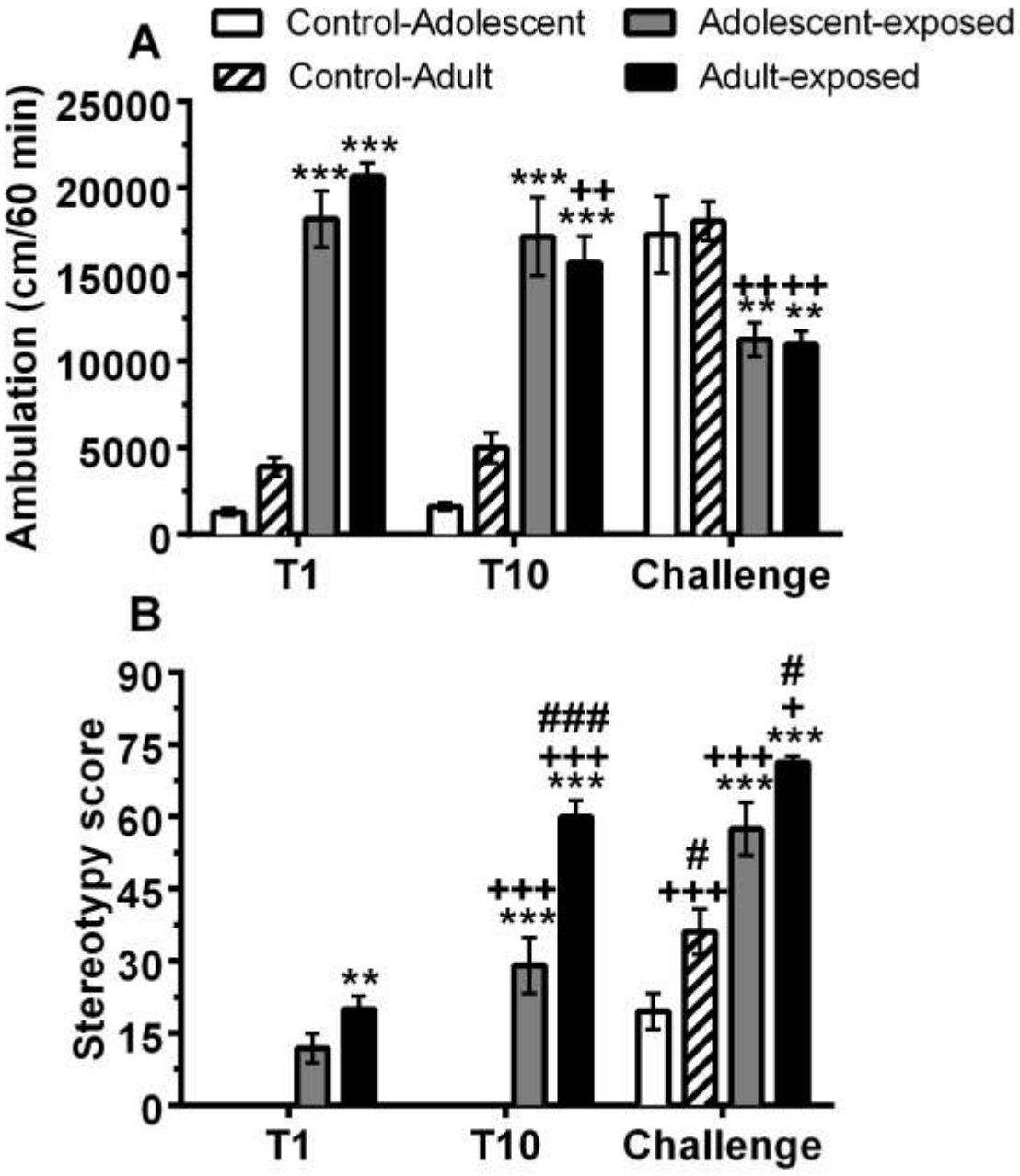


Fig. 4.2. Locomotor activity (ambulatory distance, in cm; **A**) and stereotypy rating (**B**) during the first (T1) and tenth (T10) injection of saline or AMPH and after a challenge with AMPH at the conclusion of operant testing for all rats. AMPH was always administered at a dose of 3 mg/kg (i.p.). ***p*<0.01 and ****p*<0.001, compared to age-group controls within treatment session; +*p*<0.05, ++*p*<0.01, +++*p*<0.001, compared to previous session within group; #*p*<0.05, ###*p*<0.001, between-group comparisons within session

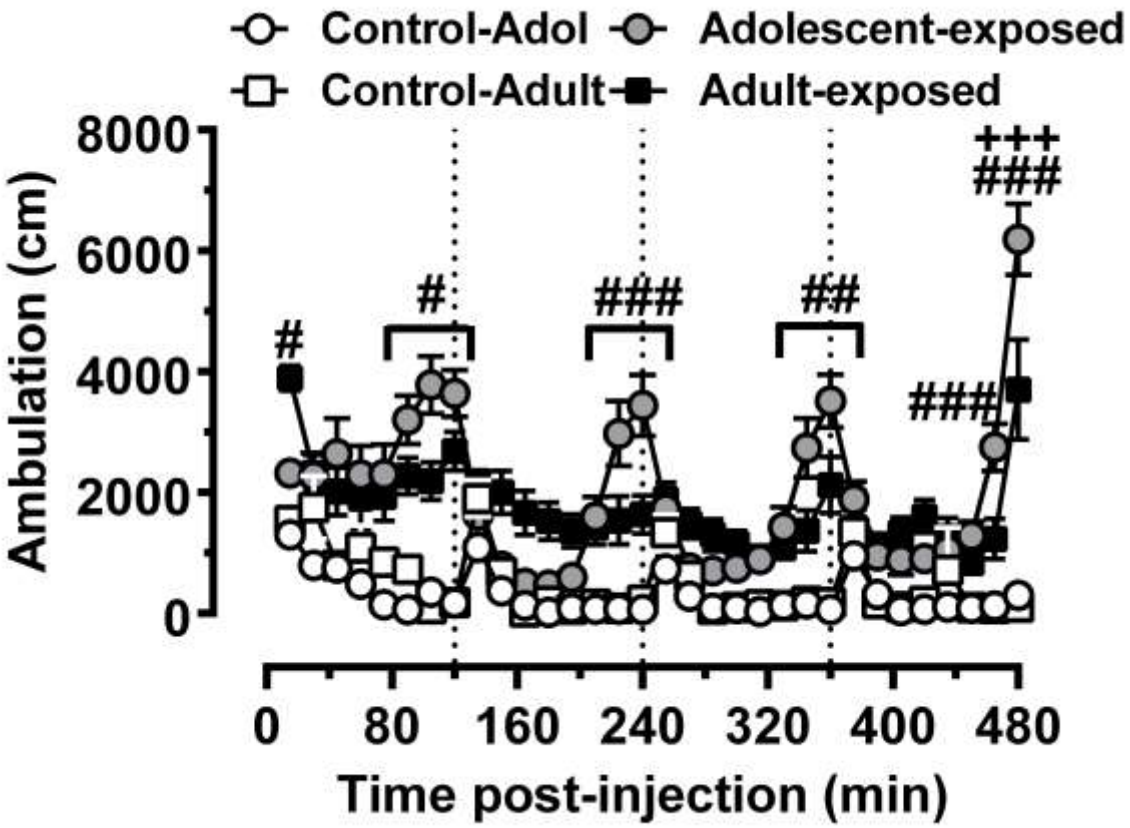


Fig. 4.3. Locomotor activity (ambulatory distance, in cm) during a post-escalation binge episode (8 hours) of saline or AMPH. Each injection (every 2 hours) is denoted by the vertical dotted lines. AMPH was always administered at a dose of 8 mg/kg (i.p.). +++p<0.001, compared to last 15 min bin of each 2-hr injection period within adolescent-exposed group; #p<0.05, ##p<0.01, ###p<0.001, between-group comparison

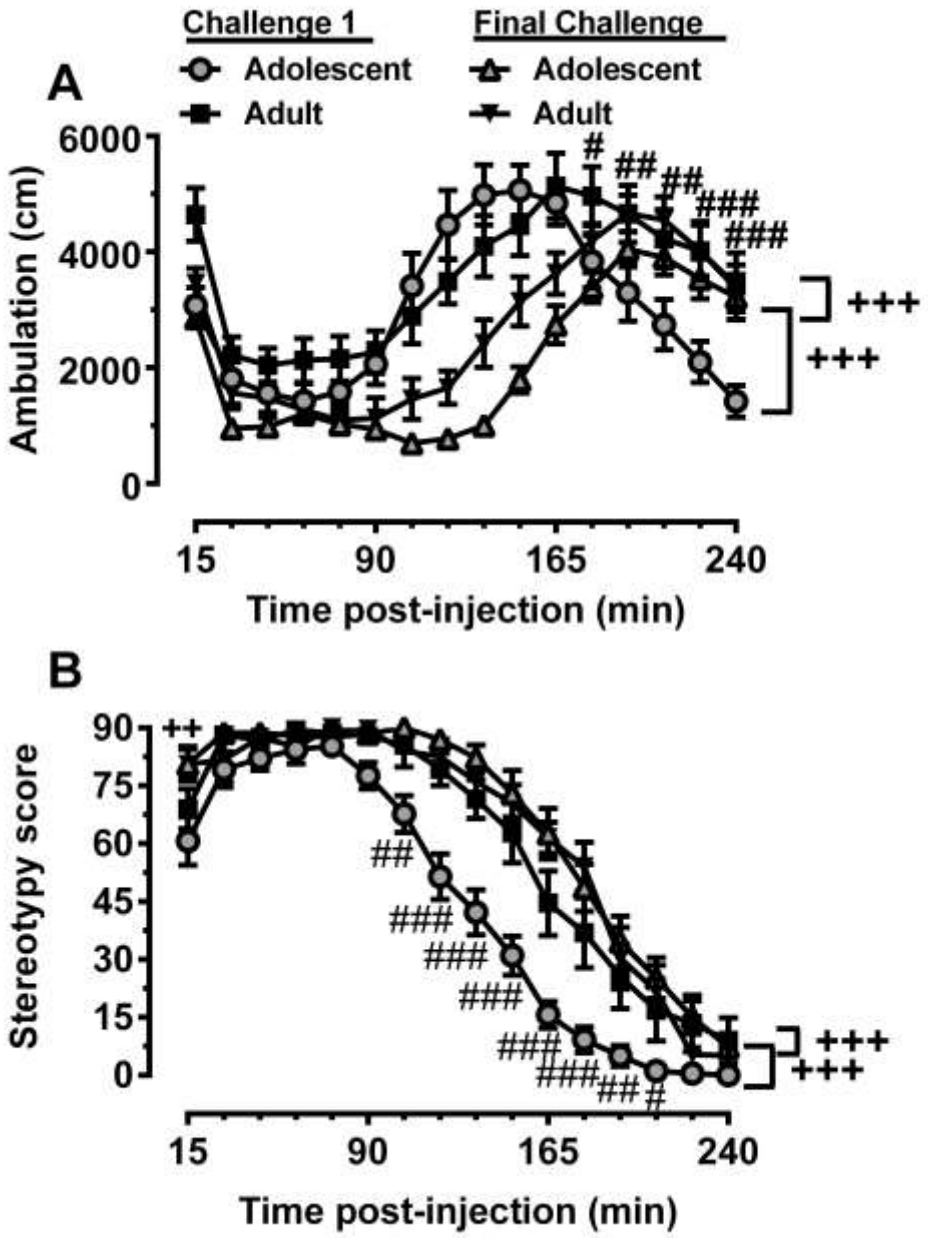


Fig. 4.4. Locomotor activity (ambulatory distance, in cm; **A**) and stereotypy rating (**B**) during the challenge session following post-escalation and binge and after a challenge with AMPH at the conclusion of operant testing for all adolescent- and adult-exposed rats. AMPH was always administered at a dose of 8 mg/kg (i.p.). +++p<0.001, compared to previous session within group; #p<0.05, ##p<0.01, ###p<0.001, between-group comparisons within challenge session

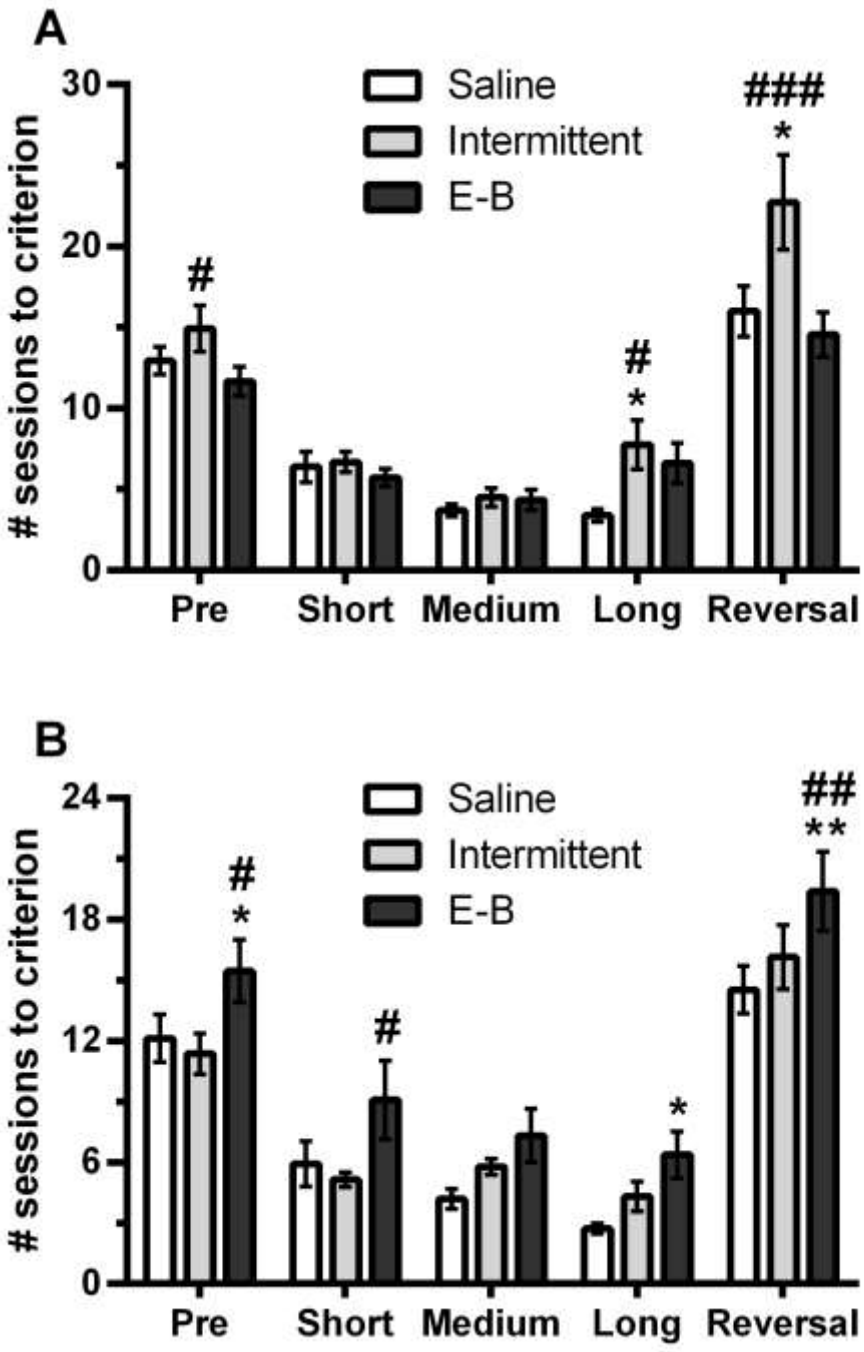


Fig. 4.5. Task criterion behavior for each delay block of the matching and non-matching (reversal) components for adolescent-exposed (**A**) and adult-exposed (**B**) rats. Data are shown as the mean number of sessions to reach ≥85% performance for two consecutive sessions. **p*<0.05 and ***p*<0.01, compared to controls within delay block; #*p*<0.05, ##*p*<0.01, ###*p*<0.001, between-age group compared to same treatment group

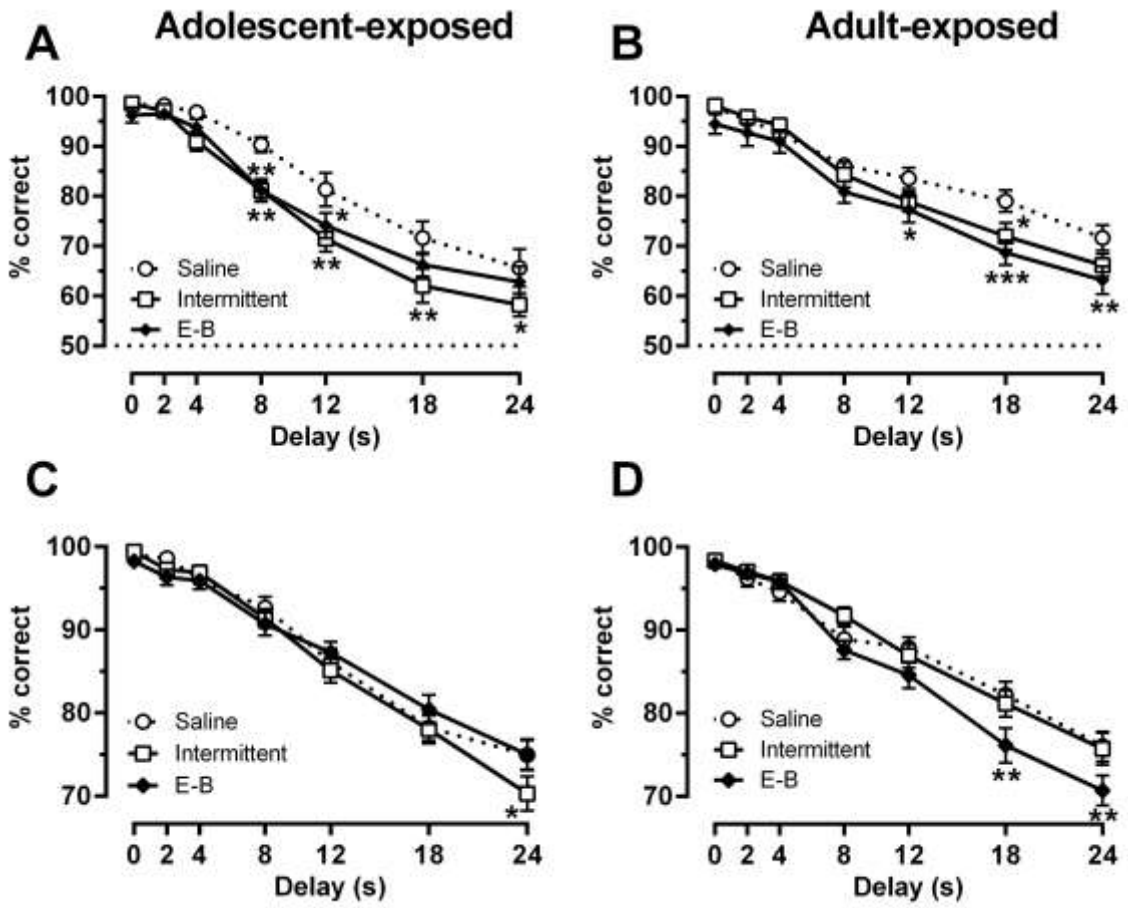


Fig. 4.6. Performance accuracy, shown as mean percent correct for treatment groups across delays, during two different phases of the long delay block (first two sessions: **A** and **B**; at criterion: **C**, **D**) for adolescent-exposed (**A**, **C**) and adult-exposed rats (**B**, **D**). **p*<0.05, ***p*<0.01, and ****p*<0.001 compared to saline controls within delay.

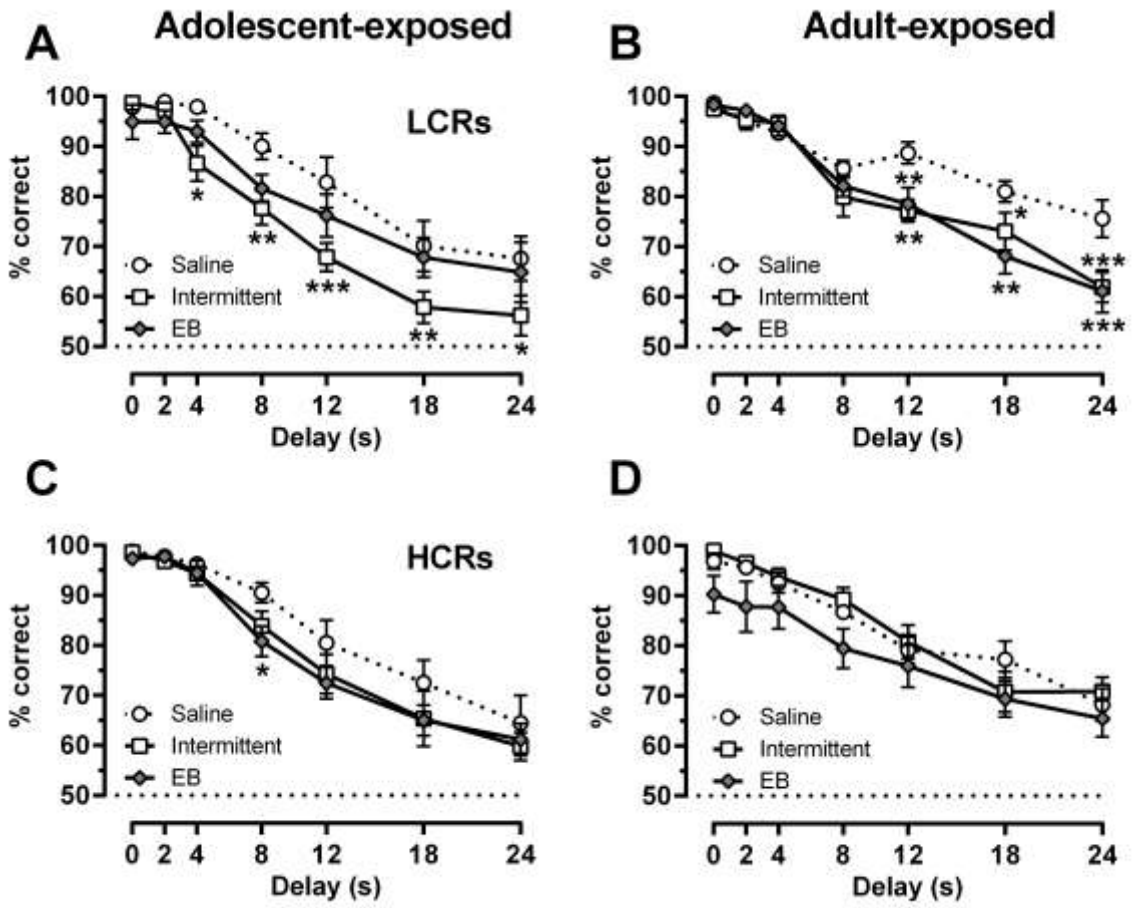


Fig. 4.7. Performance accuracy, shown as mean percent correct for treatment groups across delays, during two first two sessions of the long delay block for animals classified by their cocaine response (LCRs: **A** and **B**; HCRs: **C**, **D**) for adolescent-exposed (**A**, **C**) and adult-exposed rats (**B**, **D**). **p*<0.05, ***p*<0.01, and ****p*<0.001 compared to saline controls within delay.

	Time		
Day	8:30 am	2:00 pm	7:30 pm
1	1 mg/kg	2 mg/kg	3 mg/kg
2	3 mg/kg	4 mg/kg	5 mg/kg
3	5 mg/kg	6 mg/kg	7 mg/kg
4	7 mg/kg	8 mg/kg	
5		Day off	
6	8 mg/kg (x 4, once every 2 hours)		

Table 4.1. Treatment schedule for escalation-binge exposure to AMPH. Adapted from Segal and Kuczenski, 1997

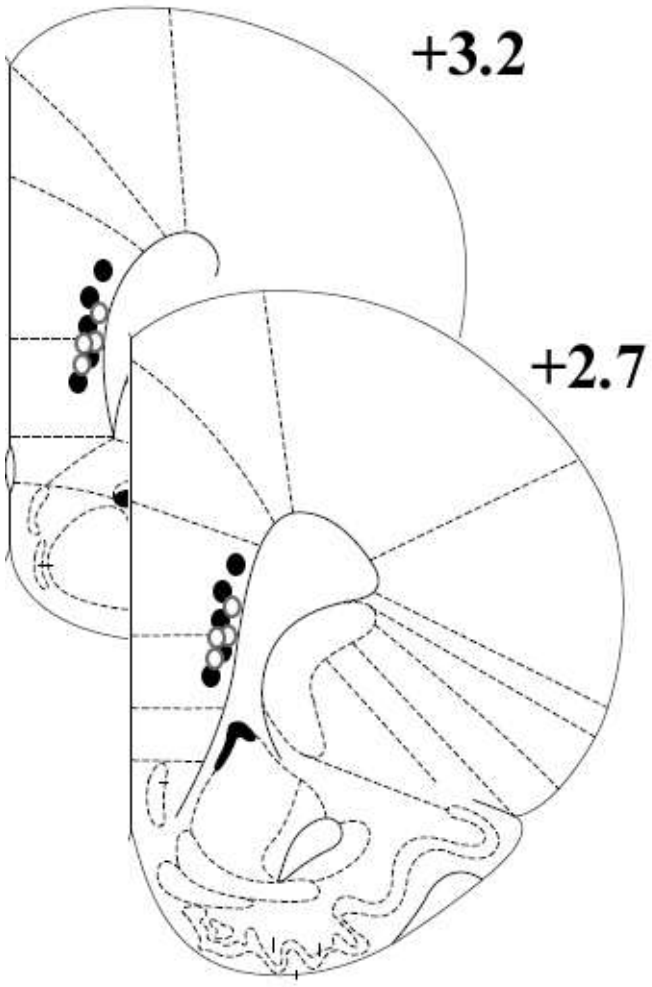


Fig. 5.1. Schematic diagram of recording site locations in the ventral mPFC. Dots represent the approximate locations of electrode microwire bundles from each rat that were histologically verified to terminate in the prelimbic and infralimbic regions. The filled dots correspond to the AMPH-treated rats (n=5), while the open dots correspond to the saline-treated rats (n=4). These coronal section images were adapted from the stereotaxic atlas of Paxinos and Watson (1998).

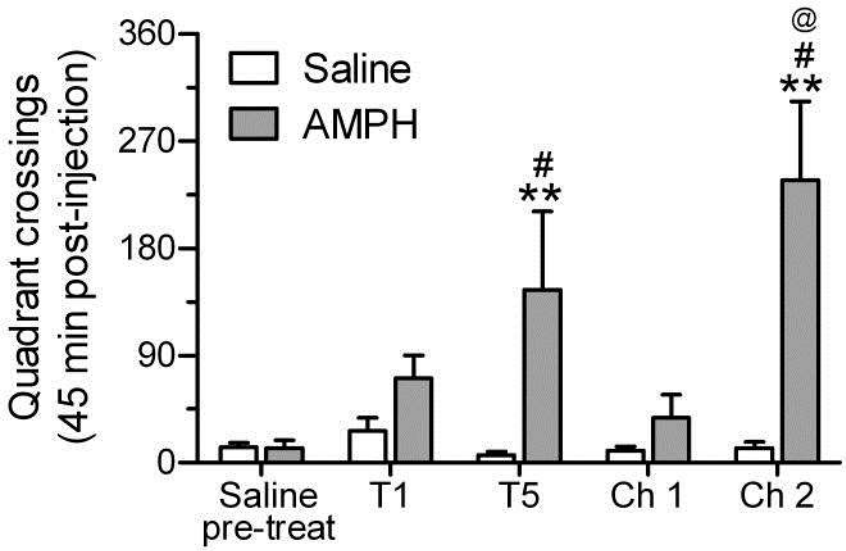


Fig. 5.2. Mean number of quadrant crossings in the open-field arena for the 45 min period after the first (T1) or fifth (T5) treatment and after challenge injections (Ch 1 and Ch 2) given following 3 days without treatment. Rats in the AMPH group (n = 5) received 1 mg/kg AMPH during tests 1 and 5 and during Ch 2; they received saline during Ch 1. Rats in the saline group (n = 4) received saline during all sessions. ** $p < 0.01$, vs. saline group within-session; # $p < 0.05$, vs. AMPH group pre-test; @ $p < 0.05$, vs. AMPH group during T1

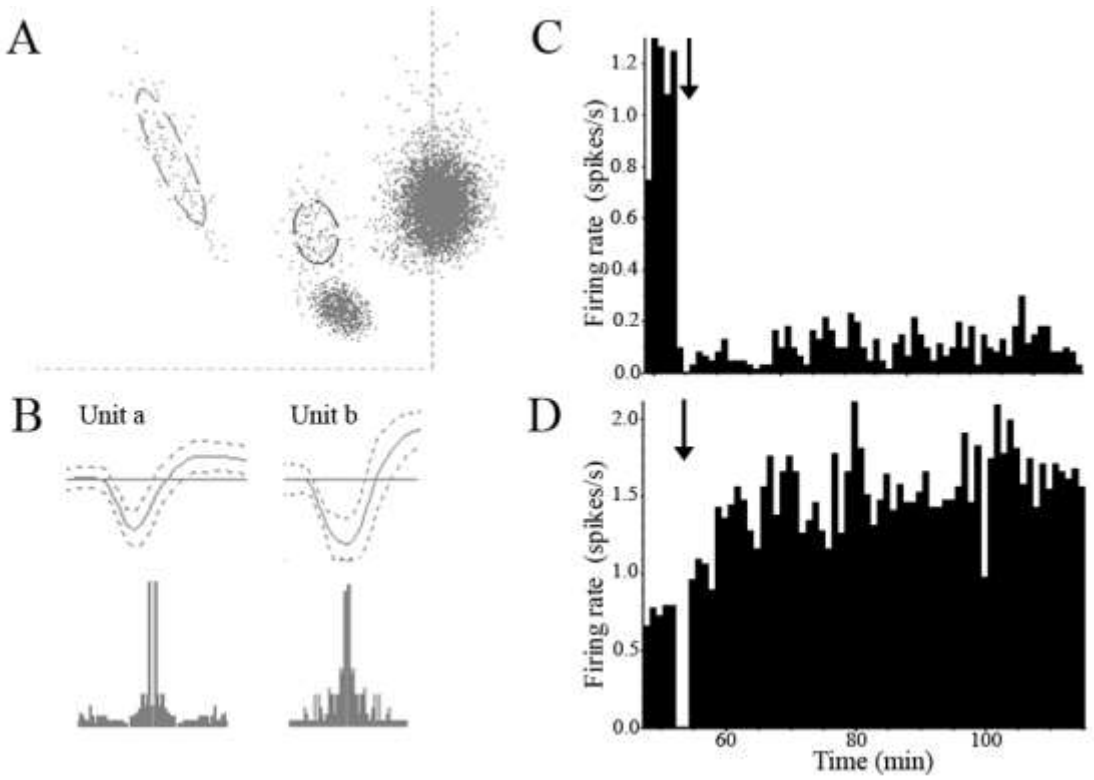


Fig. 5.3. Analysis of single-unit data recorded from microwire electrodes implanted in the rat mPFC. (**A**) 2-D scatter plot of PCA for the signal measured on one channel of a 16-channel electrode bundle. Spikes from a given neuron appear as a cluster of points, usually separated from other clusters. Cluster boundaries (represented by the dotted lines) are defined interactively during data analysis. (**B**) Waveform profiles from putative pyramidal cells recorded over the course of the experiment. 'Unit a' was recorded on first test day, while 'Unit b' was recorded 9 days later during challenge 2. Autocorrelation histograms, which are used as part of the procedure to classify single-unit activity, are also shown for these units. (**C,D**) Firing rate histograms from two different units during baseline recording and after an injection of 1 mg/kg AMPH (at arrow). The cell shown on top was classified as having a decreased firing rate in response to AMPH, whereas the cell on the bottom was classified as increased.

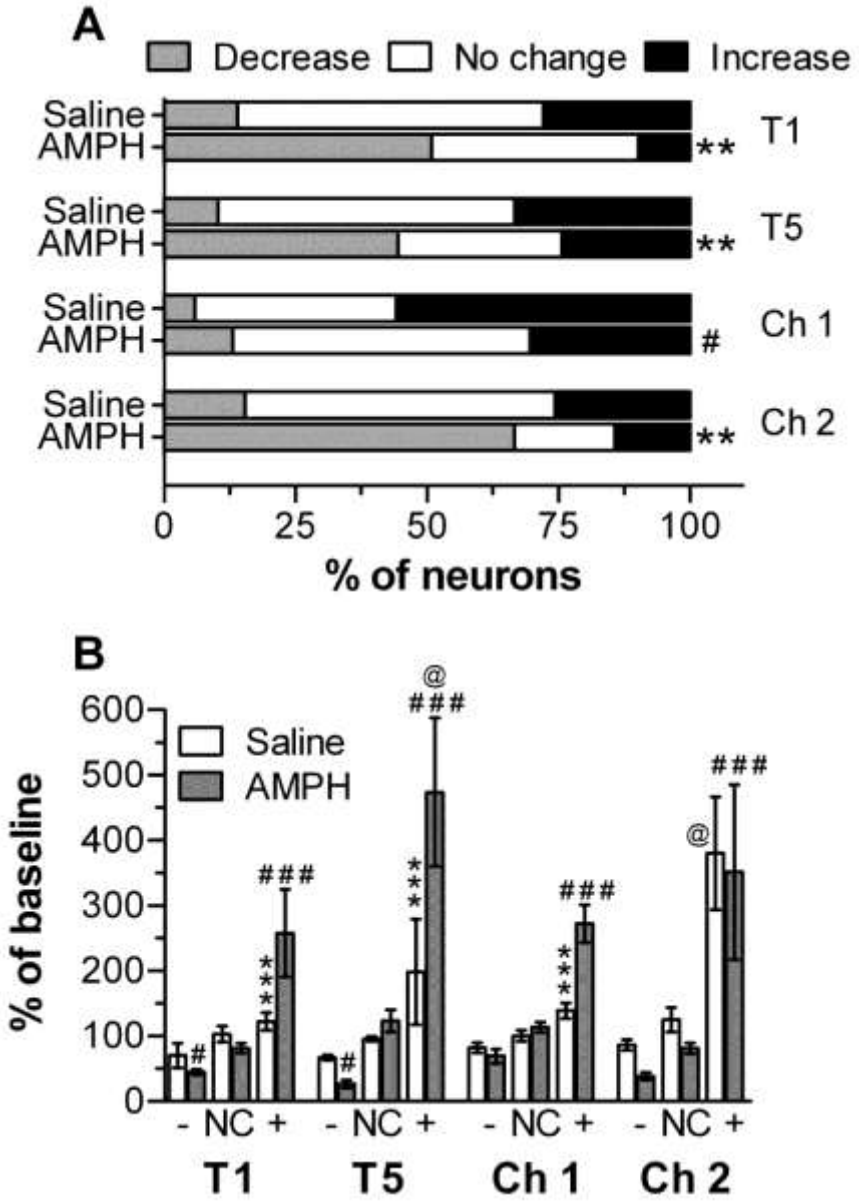


Fig. 5.4. Responses of mPFC neurons to saline and AMPH across treatment (T) and challenge (Ch) sessions. (**A**) Units were characterized as increased or decreased if mean firing rate was consistently above or below, respectively, the baseline firing rate during the 5-min period before injection (see *Experimental Procedures* for analysis details). $^{**}p < 0.001$, $\chi 2$ statistic comparing proportion of responses in AMPH and saline groups; $\#p < 0.001$, $\chi 2$ statistic comparing proportion of responses in AMPH group on Ch 1 and T1 (**B**) Firing rate, normalized to pre-injection baseline, during the 45-min period following saline or AMPH in units exhibiting a sustained decrease (-), increase (+), or no consistent change (NC). $^{*}p < 0.05$ and $^{***}p < 0.001$, compared to units increasing firing rate following AMPH injection $\#p < 0.05$ and $\#\#\#p < 0.001$, compared to units with no consistent change (NC) after AMPH injection; $@p < 0.05$, compared to firing rate during T1 for the same category of responses following the same injection type

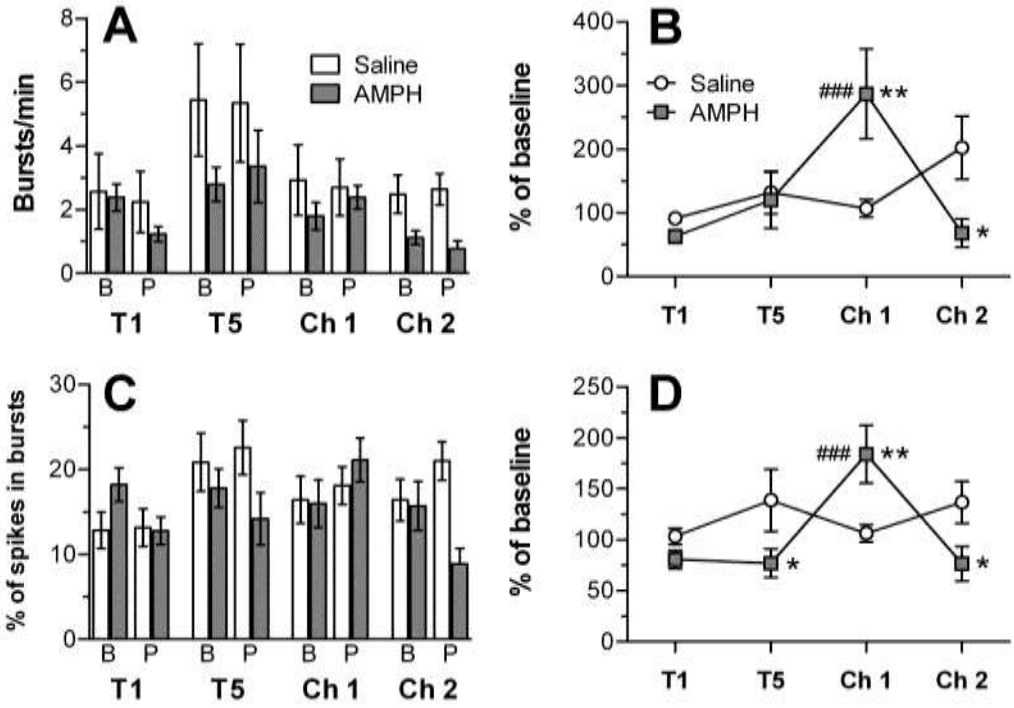


Fig. 5.5. Burst rate and percent of spikes in bursts for mPFC neurons recorded before and after injection with saline or AMPH during treatment (T) and challenge (Ch) sessions. In (**A**) and (**C**), data are presented for the baseline (B) and post-injection (P) periods. In (**B**) and (**D**), burst rate and % of spikes in bursts, respectively, are normalized to the pre-injection baseline for each unit. ###$p < 0.001$, AMPH group compared to T1; *$p < 0.05$ and **$p < 0.01$, compared to saline group

	Firing rate (spikes/s)		Burst rate (bursts/min)	
	Saline	AMPH	Saline	AMPH
Treatment 1				
(n)	(43)	(61)		
active	0.609 ± 0.136	0.605 ± 0.080	2.02 ±1.04	2.01 ± 0.44
inactive	0.650 ± 0.129	0.636 ± 0.067	2.01 ± 0.88	2.16 ± 0.31
Treatment 5				
(n)	(39)	(45)		
active	1.12 ± 0.060	0.907 ± 0.142	3.47 ± 1.52	3.65 ± 0.97
inactive	1.44 ± 0.060*	0.769 ± 0.106	5.28 ± 1.80*	2.96 ± 0.63
Challenge 1				
(n)	(34)	(46)		
active	0.659 ± 0.195	0.523 ± 0.079	2.61 ± 1.07	1.77 ± 0.43
inactive	0.644 ± 0.183	0.566 ± 0.062	2.47 ± 1.02	1.79 ± 0.36
Challenge 2				
(n)	(39)	(42)		
active	0.709 ± 0.155	0.448 ± 0.051	3.30 ± 0.96	1.15 ± 0.23
inactive	0.847 ± 0.163	0.498 ± 0.050	3.46 ± 0.86	1.32 ± 0.25

Table 5.1. Mean (± SEM) firing and burst rates for mPFC neurons recorded 30 min prior to injection with saline or AMPH on treatment days 1 and 5 and challenge days 1 and 2. Data are presented for the first and second 15-min periods that rats where in the open-field arena, which encompassed periods of high (“active”) and low (“inactive”) spontaneous motor activity, respectively. The number of cells recorded during each of the sessions (n) is shown in the firing rate columns. *$p < 0.05$, compared to active baseline

REFERENCES

Adriani W, Chiarotti F, Laviola G (1998) Elevated novelty seeking and peculiar d-amphetamine sensitization in periadolescent mice compared with adult mice. Behav Neurosci 112:1152-1166.

Ahmed SH, Koob GF (1998) Transition from moderate to excessive drug intake: change in hedonic set point. Science 282:298-300.

Allen RM, Everett CV, Nelson AM, Gulley JM, Zahniser NR (2007) Low and high locomotor responsiveness to cocaine predicts intravenous cocaine conditioned place preference in male Sprague-Dawley rats. Pharmacol Biochem Behav 86:37-44.

Andersen SL (2005) Stimulants and the developing brain. Trends Pharmacol Sci 26:237-243.

Andersen SL (2003) Trajectories of brain development: point of vulnerability or window of opportunity? Neurosci Biobehav Rev 27:3-18.

Andersen SL, Teicher MH (2008) Stress, sensitive periods and maturational events in adolescent depression. Trends Neurosci 31:183-191.

Angrist B (1994) Ethical issues regarding prospective studies of amphetamine psychosis. J Calif Alliance Ment Ill 5:32-35.

Arnsten AF, Rubia K (2012) Neurobiological circuits regulating attention, cognitive control, motivation, and emotion: disruptions in neurodevelopmental psychiatric disorders. J Am Acad Child Adolesc Psychiatry 51:356-367.

Arnsten AF, Pliszka SR (2011) Catecholamine influences on prefrontal cortical function: Relevance to treatment of attention deficit/hyperactivity disorder and related disorders. Pharmacol Biochem Behav 99:211-216.

Arnsten AF, Wang MJ, Paspalas CD (2012) Neuromodulation of thought: Flexibilities and vulnerabilities in prefrontal cortical network synapses. Neuron 76:223-239.

Badiani A, Robinson TE (2004) Drug-induced neurobehavioral plasticity: the role of environmental context. Behav Pharmacol 15:327-339.

Baeg EH, Kim YB, Huh K, Mook-Jung I, Kim HT, Jung MW (2003) Dynamics of population code for working memory in the prefrontal cortex. Neuron 40:177-188.

Ball KT, Budreau D, Rebec GV (2006) Context-dependent behavioural and neuronal sensitization in striatum to MDMA (ecstasy) administration in rats. Eur J Neurosci 24:217-228.

Barbelivien A, Billy E, Lazarus C, Kelche C, Majchrzak M (2008) Rats with different profiles of impulsive choice behavior exhibit differences in responses to caffeine and d-amphetamine and in medial prefrontal cortex 5-HT utilization. Behav Brain Res 187:273-283.

Bardo MT (1998) Neuropharmacological mechanisms of drug reward: beyond dopamine in the nucleus accumbens. Crit Rev Neurobiol 12:37-67.

Bardo MT, Cain ME, Bylica KE (2006) Effect of amphetamine on response inhibition in rats showing high or low response to novelty. Pharmacol Biochem Behav 85:98-104.

Bartho P, Hirase H, Monconduit L, Zugaro M, Harris KD, Buzsaki G (2004) Characterization of neocortical principal cells and interneurons by network interactions and extracellular features. J Neurophysiol 92:600-608.

Bartlett E, Hallin A, Chapman B, Angrist B (1997) Selective sensitization to the psychosis-inducing effects of cocaine: a possible marker for addiction relapse vulnerability? Neuropsychopharmacology 16:77-82.

Bast T, Pezze MA, Feldon J (2002) Dopamine receptor blockade in the rat medial prefrontal cortex reduces spontaneous and amphetamine-induced activity and does not affect prepulse inhibition. Behav Pharmacol 13:669-673.

Bechara A (2005) Decision making, impulse control and loss of willpower to resist drugs: a neurocognitive perspective. Nat Neurosci 8:1458-1463.

Bechara A, Damasio AR, Damasio H, Anderson SW (1994) Insensitivity to future consequences following damage to human prefrontal cortex. Cognition 50:7-15.

Belcher AM, Feinstein EM, O'Dell SJ, Marshall JF (2008) Methamphetamine influences on recognition memory: comparison of escalating and single-day dosing regimens. Neuropsychopharmacology 33:1453-1463.

Belcher AM, O'Dell SJ, Marshall JF (2006) A sensitizing regimen of methamphetamine causes impairments in a novelty preference task of object recognition. Behav Brain Res 170:167-172.

Bell HC, Pellis SM, Kolb B (2010) Juvenile peer play experience and the development of the orbitofrontal and medial prefrontal cortices. Behav Brain Res 207:7-13.

Ben-Shahar O, Ahmed SH, Koob GF, Ettenberg A (2004) The transition from controlled to compulsive drug use is associated with a loss of sensitization. Brain Res 995:46-54.

Ben-Shahar O, Moscarello JM, Jacob B, Roarty MP, Ettenberg A (2005) Prolonged daily exposure to i.v. cocaine results in tolerance to its stimulant effects. Pharmacol Biochem Behav 82:411-416.

Berman KF, Weinberger DR (1990) The prefrontal cortex in schizophrenia and other neuropsychiatric diseases: in vivo physiological correlates of cognitive deficits. Prog Brain Res 85:521-36; discussion 536-7.

Berman SM, Kuczenski R, McCracken JT, London ED (2009) Potential adverse effects of amphetamine treatment on brain and behavior: a review. Mol Psychiatry 14:123-142.

Bickel WK, Marsch LA (2001) Toward a behavioral economic understanding of drug dependence: delay discounting processes. Addiction 96:73-86.

Bisagno V, Ferguson D, Luine VN (2002) Short toxic methamphetamine schedule impairs object recognition task in male rats. Brain Res 940:95-101.

Bisagno V, Ferguson D, Luine VN (2003) Chronic D-amphetamine induces sexually dimorphic effects on locomotion, recognition memory, and brain monoamines. Pharmacol Biochem Behav 74:859-867.

Bizot J, Le Bihan C, Puech AJ, Hamon M, Thiebot M (1999) Serotonin and tolerance to delay of reward in rats. Psychopharmacology (Berl) 146:400-412.

Bjijou Y, De Deurwaerdere P, Spampinato U, Stinus L, Cador M (2002) D-amphetamine-induced behavioral sensitization: effect of lesioning dopaminergic terminals in the medial prefrontal cortex, the amygdala and the entorhinal cortex. Neuroscience 109:499-516.

Boileau I, Dagher A, Leyton M, Gunn RN, Baker GB, Diksic M, Benkelfat C (2006) Modeling sensitization to stimulants in humans: an [11C]raclopride/positron emission tomography study in healthy men. Arch Gen Psychiatry 63:1386-1395.

Bolanos CA, Barrot M, Berton O, Wallace-Black D, Nestler EJ (2003) Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood. Biol Psychiatry 54:1317-1329.

Bolanos CA, Glatt SJ, Jackson D (1998) Subsensitivity to dopaminergic drugs in periadolescent rats: a behavioral and neurochemical analysis. Brain Res Dev Brain Res 111:25-33.

Borgland SL, Malenka RC, Bonci A (2004) Acute and chronic cocaine-induced potentiation of synaptic strength in the ventral tegmental area: electrophysiological and behavioral correlates in individual rats. J Neurosci 24:7482-7490.

Bornovalova MA, Daughters SB, Hernandez GD, Richards JB, Lejuez CW (2005) Differences in impulsivity and risk-taking propensity between primary users of crack cocaine and primary users of heroin in a residential substance-use program. Exp Clin Psychopharmacol 13:311-318.

Bouret S, Sara SJ (2004) Reward expectation, orientation of attention and locus coeruleus-medial frontal cortex interplay during learning. Eur J Neurosci 20:791-802.

Brandon CL, Marinelli M, Baker LK, White FJ (2001) Enhanced reactivity and vulnerability to cocaine following methylphenidate treatment in adolescent rats. Neuropsychopharmacology 25:651-661.

Brenhouse HC, Andersen SL (2011) Developmental trajectories during adolescence in males and females: a cross-species understanding of underlying brain changes. Neurosci Biobehav Rev 35:1687-1703.

Brenhouse HC, Sonntag KC, Andersen SL (2008) Transient D1 dopamine receptor expression on prefrontal cortex projection neurons: Relationship to enhanced motivational salience of drug cues in adolescence. J Neurosci 28:2375-2382.

Briand LA, Robinson TE, Maren S (2005) Enhancement of auditory fear conditioning after housing in a complex environment is attenuated by prior treatment with amphetamine. Learn Mem 12:553-556.

Briegleb SK, Gulley JM, Hoover BR, Zahniser NR (2004) Individual differences in cocaine- and amphetamine-induced activation of male Sprague-Dawley rats: contribution of the dopamine transporter. Neuropsychopharmacology 29:2168-2179.

Brown VJ, Bowman EM (2002) Rodent models of prefrontal cortical function. Trends Neurosci 25:340-343.

Brozoski TJ, Brown RM, Rosvold HE, Goldman PS (1979) Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. Science 205:929-932.

Bubar MJ, Cunningham KA (2006) Serotonin 5-HT2A and 5-HT2C receptors as potential targets for modulation of psychostimulant use and dependence. Curr Top Med Chem 6:1971-1985.

Burgos-Robles A, Vidal-Gonzalez I, Santini E, Quirk GJ (2007) Consolidation of fear extinction requires NMDA receptor-dependent bursting in the ventromedial prefrontal cortex. Neuron 53:871-880.

Buzsaki G, Csicsvari J, Dragoi G, Harris K, Henze D, Hirase H (2002) Homeostatic maintenance of neuronal excitability by burst discharges in vivo. Cereb Cortex 12:893-899.

Cador M, Bjijou Y, Cailhol S, Stinus L (1999) D-amphetamine-induced behavioral sensitization: implication of a glutamatergic medial prefrontal cortex-ventral tegmental area innervation. Neuroscience 94:705-721.

Cardinal RN, Robbins TW, Everitt BJ (2000) The effects of d-amphetamine, chlordiazepoxide, alpha-flupenthixol and behavioural manipulations on choice of signalled and unsignalled delayed reinforcement in rats. Psychopharmacology (Berl) 152:362-375.

Carey RJ, De Palma G, Damianopoulos E (2002) 5-HT1A agonist/antagonist modification of cocaine stimulant effects: implications for cocaine mechanisms. Behav Brain Res 132:37-46.

Carey RJ, DePalma G, Damianopoulos E (2001) Cocaine and serotonin: a role for the 5-HT(1A) receptor site in the mediation of cocaine stimulant effects. Behav Brain Res 126:127-133.

Carey RJ, Depalma G, Damianopoulos E, Muller CP, Huston JP (2004) The 5-HT1A receptor and behavioral stimulation in the rat: effects of 8-OHDPAT on spontaneous and cocaine-induced behavior. Psychopharmacology (Berl) 177:46-54.

Carey RJ, DePalma G, Damianopoulos E, Shanahan A., Muller CP, Huston JP (2005) Evidence that the 5-HT1A autoreceptor is an important pharmacological target for the modulation of cocaine behavioral stimulant effects. Brain Res 1034:162-171.

Carlezon WA,Jr, Mague SD, Andersen SL (2003) Enduring behavioral effects of early exposure to methylphenidate in rats. Biol Psychiatry 54:1330-1337.

Casey BJ, Tottenham N, Liston C, Durston S (2005) Imaging the developing brain: what have we learned about cognitive development? Trends Cogn Sci 9:104-110.

Castner SA, Vosler PS, Goldman-Rakic PS (2005) Amphetamine sensitization impairs cognition and reduces dopamine turnover in primate prefrontal cortex. Biol Psychiatry 57:743-751.

Chang L, Alicata D, Ernst T, Volkow N (2007) Structural and metabolic brain changes in the striatum associated with methamphetamine abuse. Addiction 102 Suppl 1:16-32.

Chen BT, Bowers MS, Martin M, Hopf FW, Guillory AM, Carelli RM, Chou JK, Bonci A (2008) Cocaine but not natural reward self-administration nor passive cocaine infusion produces persistent LTP in the VTA. Neuron 59:288-297.

Chen NH, Reith ME (1995) Monoamine interactions measured by microdialysis in the ventral tegmental area of rats treated systemically with (+/-)-8-hydroxy-2-(di-n-propylamino)tetralin. J Neurochem 64:1585-1597.

Childress AR, Mozley PD, McElgin W, Fitzgerald J, Reivich M, O'Brien CP (1999) Limbic activation during cue-induced cocaine craving. Am J Psychiatry 156:11-18.

Cho AK, Melega WP, Kuczenski R, Segal DS (2001) Relevance of pharmacokinetic parameters in animal models of methamphetamine abuse. Synapse 39:161-166.

Chudasama Y, Muir JL (1997) A behavioural analysis of the delayed non-matching to position task: the effects of scopolamine, lesions of the fornix and of the prelimbic region on mediating behaviours by rats. Psychopharmacology (Berl) 134:73-82.

Clark RE, Kuczenski R, Segal DS (2007) Escalating dose, multiple binge methamphetamine regimen does not impair recognition memory in rats. Synapse 61:515-522.

Coffey SF, Gudleski GD, Saladin ME, Brady KT (2003) Impulsivity and rapid discounting of delayed hypothetical rewards in cocaine-dependent individuals. Exp Clin Psychopharmacol 11:18-25.

Cole BJ, Robbins TW (1989) Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi on performance of a 5-choice serial reaction time task in rats: Implications for theories of selective attention and arousal. Behav Brain Res 33:165-179.

Connors BW, Gutnick MJ (1990) Intrinsic firing patterns of diverse neocortical neurons. Trends Neurosci 13:99-104.

Cowen SL, McNaughton BL (2007) Selective delay activity in the medial prefrontal cortex of the rat: contribution of sensorimotor information and contingency. J Neurophysiol 98:303-316.

Crombag HS, Gorny G, Li Y, Kolb B, Robinson TE (2005) Opposite effects of amphetamine self-administration experience on dendritic spines in the medial and orbital prefrontal cortex. Cereb Cortex 15:341-348.

Cunningham MG, Bhattacharyya S, Benes FM (2002) Amygdalo-cortical sprouting continues into early adulthood: implications for the development of normal and abnormal function during adolescence. J Comp Neurol 453:116-130.

Dalley JW, Everitt BJ, Robbins TW (2011) Impulsivity, compulsivity, and top-down cognitive control. Neuron 69:680-694.

Dalley JW, Fryer TD, Brichard L, Robinson ES, Theobald DE, Laane K, Pena Y, Murphy ER, Shah Y, Probst K, Abakumova I, Aigbirhio FI, Richards HK, Hong Y, Baron

JC, Everitt BJ, Robbins TW (2007a) Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. Science 315:1267-1270.

Dalley JW, Laane K, Theobald DE, Pena Y, Bruce CC, Huszar AC, Wojcieszek M, Everitt BJ, Robbins TW (2007b) Enduring deficits in sustained visual attention during withdrawal of intravenous methylenedioxymethamphetamine self-administration in rats: results from a comparative study with d-amphetamine and methamphetamine. Neuropsychopharmacology 32:1195-1206.

Dalley JW, Mar AC, Economidou D, Robbins TW (2008) Neurobehavioral mechanisms of impulsivity: fronto-striatal systems and functional neurochemistry. Pharmacol Biochem Behav 90(2):250-260.

Dalley JW, Theobald DE, Berry D, Milstein JA, Laane K, Everitt BJ, Robbins TW (2005) Cognitive sequelae of intravenous amphetamine self-administration in rats: evidence for selective effects on attentional performance. Neuropsychopharmacology 30:525-537.

Davidson ES, Finch JF, Schenk S (1993) Variability in subjective responses to cocaine: initial experiences of college students. Addict Behav 18:445-453.

De La Garza R,2nd, Cunningham KA (2000) The effects of the 5-hydroxytryptamine(1A) agonist 8-hydroxy-2-(di-n-propylamino)tetralin on spontaneous activity, cocaine-induced hyperactivity and behavioral sensitization: a microanalysis of locomotor activity. J Pharmacol Exp Ther 292:610-617.

Delatour B, Gisquet-Verrier P (2000) Functional role of rat prelimbic-infralimbic cortices in spatial memory: evidence for their involvement in attention and behavioural flexibility. Behav Brain Res 109:113-128.

DeWit DJ, Offord DR, Wong M (1997) Patterns of onset and cessation of drug use over the early part of the life course. Health Educ Behav 24:746-758.

de Wit H, Enggasser JL, Richards JB (2002) Acute administration of d-amphetamine decreases impulsivity in healthy volunteers. Neuropsychopharmacology 27:813-825.

Deller T, Sarter M (1998) Effects of repeated administration of amphetamine on behavioral vigilance: evidence for "sensitized" attentional impairments. Psychopharmacology (Berl) 137:410-414.

Dellu-Hagedorn F (2006) Relationship between impulsivity, hyperactivity and working memory: a differential analysis in the rat. Behav Brain Funct 2:10.

Dias R, Aggleton JP (2000) Effects of selective excitotoxic prefrontal lesions on acquisition of nonmatching- and matching-to-place in the T-maze in the rat: Differential involvement of the prelimbic-infralimbic and anterior cingulate cortices in providing behavioural flexibility. Eur J Neurosci 12:4457-4466.

Diaz Heijtz R, Kolb B, Forssberg H (2003) Can a therapeutic dose of amphetamine during pre-adolescence modify the pattern of synaptic organization in the brain? Eur J Neurosci 18:3394-3399.

Dolan SL, Bechara A, Nathan PE (2008) Executive dysfunction as a risk marker for substance abuse: The role of impulsive personality traits. Behav Sci Law 26:799-822.

Dong Y, Nasif FJ, Tsui JJ, Ju WY, Cooper DC, Hu XT, Malenka RC, White FJ (2005) Cocaine-induced plasticity of intrinsic membrane properties in prefrontal cortex pyramidal neurons: adaptations in potassium currents. J Neurosci 25:936-940.

Dunnett SB (1985) Comparative effects of cholinergic drugs and lesions of nucleus basalis or fimbria-fornix on delayed matching in rats. Psychopharmacology (Berl) 87:357-363.

Dunnett SB, Bunch ST, Gage FH, Bjorklund A (1984) Dopamine-rich transplants in rats with 6-OHDA lesions of the ventral tegmental area. I. Effects on spontaneous and drug-induced locomotor activity. Behav Brain Res 13:71-82.

Eagle DM, Tufft MR, Goodchild HL, Robbins TW (2007) Differential effects of modafinil and methylphenidate on stop-signal reaction time task performance in the rat, and interactions with the dopamine receptor antagonist cis-flupenthixol. Psychopharmacology (Berl) 192:193-206.

Economidou D, Theobald DE, Robbins TW, Everitt BJ, Dalley JW (2012) Norepinephrine and dopamine modulate impulsivity on the five-choice serial reaction time task through opponent actions in the shell and core sub-regions of the nucleus accumbens. Neuropsychopharmacology 37:2057-2066.

Ernst M, Fudge JL (2009) A developmental neurobiological model of motivated behavior: anatomy, connectivity and ontogeny of the triadic nodes. Neurosci Biobehav Rev 33:367-382.

Ersche KD, Clark L, London M, Robbins TW, Sahakian BJ (2006) Profile of executive and memory function associated with amphetamine and opiate dependence. Neuropsychopharmacology 31:1036-1047.

Ersche KD, Roiser JP, Robbins TW, Sahakian BJ (2008) Chronic cocaine but not chronic amphetamine use is associated with perseverative responding in humans. Psychopharmacology (Berl) 197(3):421-431.

Ersche KD, Turton AJ, Chamberlain SR, Muller U, Bullmore ET, Robbins TW (2012) Cognitive dysfunction and anxious-impulsive personality traits are endophenotypes for drug dependence. Am J Psychiatry 169:926-936.

Ersche KD, Barnes A, Jones PS, Morein-Zamir S, Robbins TW, Bullmore ET (2011) Abnormal structure of frontostriatal brain systems is associated with aspects of impulsivity and compulsivity in cocaine dependence. Brain 134:2013-2024.

Euston DR, McNaughton BL (2006) Apparent encoding of sequential context in rat medial prefrontal cortex is accounted for by behavioral variability. J Neurosci 26:13143-13155.

Evenden JL (1999) Varieties of impulsivity. Psychopharmacology (Berl) 146:348-361.

Evenden JL, Ryan CN (1999) The pharmacology of impulsive behaviour in rats VI: the effects of ethanol and selective serotonergic drugs on response choice with varying delays of reinforcement. Psychopharmacology (Berl) 146:413-421.

Evenden JL, Ryan CN (1996) The pharmacology of impulsive behaviour in rats: the effects of drugs on response choice with varying delays of reinforcement. Psychopharmacology (Berl) 128:161-170.

Everitt BJ, Robbins TW (2013) From the ventral to the dorsal striatum: Devolving views of their roles in drug addiction. Neurosci Biobehav Rev.

Everitt BJ, Wolf ME (2002) Psychomotor stimulant addiction: A neural systems perspective. J Neurosci 22:3312-3320.

Fanous S, Lacagnina MJ, Nikulina EM, Hammer RP,Jr (2011) Sensitized activation of fos and brain-derived neurotrophic factor in the medial prefrontal cortex and ventral tegmental area accompanies behavioral sensitization to amphetamine. Neuropharmacology 61:558-564.

Featherstone RE, Rizos Z, Kapur S, Fletcher PJ (2008) A sensitizing regimen of amphetamine that disrupts attentional set-shifting does not disrupt working or long-term memory. Behav Brain Res 189:170-179.

Fleckenstein AE, Gibb JW, Hanson GR (2000) Differential effects of stimulants on monoaminergic transporters: pharmacological consequences and implications for neurotoxicity. Eur J Pharmacol 406:1-13.

Fletcher PJ, Tenn CC, Rizos Z, Lovic V, Kapur S (2005) Sensitization to amphetamine, but not PCP, impairs attentional set shifting: reversal by a D1 receptor agonist injected into the medial prefrontal cortex. Psychopharmacology (Berl) 183:190-200.

Fletcher PJ, Tenn CC, Sinyard J, Rizos Z, Kapur S (2007) A sensitizing regimen of amphetamine impairs visual attention in the 5-choice serial reaction time test: reversal by a D1 receptor agonist injected into the medial prefrontal cortex. Neuropsychopharmacology 32:1122-1132.

Floresco SB, Magyar O (2006) Mesocortical dopamine modulation of executive functions: Beyond working memory. Psychopharmacology (Berl) 188:567-585.

Floresco SB, Tse MT, Ghods-Sharifi S (2007) Dopaminergic and glutamatergic regulation of effort- and delay-based decision making. Neuropsychopharmacology 33(8):1966-1979.

Ford KA, Wolf ME, Hu XT (2009) Plasticity of L-type Ca2+ channels after cocaine withdrawal. Synapse 63:690-697.

Franklin TR, Acton PD, Maldjian JA, Gray JD, Croft JR, Dackis CA, O'Brien CP, Childress AR (2002) Decreased gray matter concentration in the insular, orbitofrontal, cingulate, and temporal cortices of cocaine patients. Biol Psychiatry 51:134-142.

Fuster JM, Alexander GE (1971) Neuron activity related to short-term memory. Science 173:652-654.

Garavan H, Pankiewicz J, Bloom A, Cho JK, Sperry L, Ross TJ, Salmeron BJ, Risinger R, Kelley D, Stein EA (2000) Cue-induced cocaine craving: neuroanatomical specificity for drug users and drug stimuli. Am J Psychiatry 157:1789-1798.

Gawin FH, Khalsa-Denison ME (1996) Is craving mood-driven or self-propelled? Sensitization and "street" stimulant addiction. NIDA Res Monogr 163:224-250.

Gawin FH (1991) Cocaine addiction: psychology and neurophysiology. Science 251:1580-1586.

Gemmell C, Anderson M, O'Mara SM (2002) Deep layer prefrontal cortex unit discharge in a cue-controlled open-field environment in the freely-moving rat. Behav Brain Res 133:1-10.

George O, Mandyam CD, Wee S, Koob GF (2008) Extended access to cocaine self-administration produces long-lasting prefrontal cortex-dependent working memory impairments. Neuropsychopharmacology 33(10):2474-2482.

Goldman-Rakic PS (1995) Architecture of the prefrontal cortex and the central executive. Ann N Y Acad Sci 769:71-83.

Goldstein RZ, Volkow ND (2002) Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. Am J Psychiatry 159:1642-1652.

Granon S, Vidal C, Thinus-Blanc C, Changeux JP, Poucet B (1994) Working memory, response selection, and effortful processing in rats with medial prefrontal lesions. Behav Neurosci 108:883-891.

Graybiel AM, Moratalla R, Robertson HA (1990) Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. Proc Natl Acad Sci U S A 87:6912-6916.

Greenough WT, Chang FL (1988) Dendritic pattern formation involves both oriented regression and oriented growth in the barrels of mouse somatosensory cortex. Brain Res 471:148-152.

Gulley JM (2007) Individual differences in novelty- and cocaine-induced locomotor activity as predictors of food-reinforced operant behavior in two outbred rat strains. Pharmacol Biochem Behav 86:749-757.

Gulley JM, Hoover BR, Larson GA, Zahniser NR (2003) Individual differences in cocaine-induced locomotor activity in rats: behavioral characteristics, cocaine pharmacokinetics, and the dopamine transporter. Neuropsychopharmacology 28:2089-2101.

Gulley JM, Kosobud AE, Rebec GV (2002) Behavior-related modulation of substantia nigra pars reticulate neurons in rats performing a conditioned reinforcement task. Neuroscience 111:337-349.

Gulley JM, Reed JL, Kuwajima M, Rebec GV (2004) Amphetamine-induced behavioral activation is associated with variable changes in basal ganglia output neurons recorded from awake, behaving rats. Brain Res 1012:108-118.

Haertzen CA, Kocher TR, Miyasato K (1983) Reinforcements from the first drug experience can predict later drug habits and/or addiction: results with coffee, cigarettes, alcohol, barbiturates, minor and major tranquilizers, stimulants, marijuana, hallucinogens, heroin, opiates and cocaine. Drug Alcohol Depend 11:147-165.

Hall DA, Powers JP, Gulley JM (2009) Blockade of D1 dopamine receptors in the medial prefrontal cortex attenuates amphetamine- and methamphetamine-induced locomotor activity in the rat. Brain Res 1300:51-57.

Hall DA, Stanis JJ, Marquez Avila H, Gulley JM (2008) A comparison of amphetamine- and methamphetamine-induced locomotor activity in rats: evidence for qualitative differences in behavior. Psychopharmacology (Berl) 195:469-478.

Hankosky ER, Gulley JM (2012) Performance on an impulse control task is altered in adult rats exposed to amphetamine during adolescence. Dev Psychobiol.

Harris KD, Hirase H, Leinekugel X, Henze DA, Buzsaki G (2001) Temporal interaction between single spikes and complex spike bursts in hippocampal pyramidal cells. Neuron 32:141-149.

Harvey RC, Dembro KA, Rajagopalan K, Mutebi MM, Kantak KM (2009) Effects of self-administered cocaine in adolescent and adult male rats on orbitofrontal cortex-related neurocognitive functioning. Psychopharmacology (Berl) 206:61-71.

Hedou G, Jongen-Relo AL, Murphy CA, Heidbreder CA, Feldon J (2002) Sensitized fos expression in subterritories of the rat medial prefrontal cortex and nucleus accumbens following amphetamine sensitization as revealed by stereology. Brain Res 950:165-179.

Heidbreder CA, Groenewegen HJ (2003) The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. Neurosci Biobehav Rev 27:555-579.

Heil SH, Johnson MW, Higgins ST, Bickel WK (2006) Delay discounting in currently using and currently abstinent cocaine-dependent outpatients and non-drug-using matched controls. Addict Behav 31:1290-1294.

Herges S, Taylor DA (1998) Involvement of serotonin in the modulation of cocaine-induced locomotor activity in the rat. Pharmacol Biochem Behav 59:595-611.

Ho MY, Velazquez-Martinez DN, Bradshaw CM, Szabadi E (2002) 5-Hydroxytryptamine and interval timing behaviour. Pharmacol Biochem Behav 71:773-785.

Hoffman WF, Moore M, Templin R, McFarland B, Hitzemann RJ, Mitchell SH (2006) Neuropsychological function and delay discounting in methamphetamine-dependent individuals. Psychopharmacology (Berl) 188:162-170.

Homayoun H, Moghaddam B (2006) Progression of cellular adaptations in medial prefrontal and orbitofrontal cortex in response to repeated amphetamine. J Neurosci 26:8025-8039.

Hotsenpiller G, Giorgetti M, Wolf ME (2001) Alterations in behaviour and glutamate transmission following presentation of stimuli previously associated with cocaine exposure. Eur J Neurosci 14:1843-1855.

Huttenlocher PR (1984) Synapse elimination and plasticity in developing human cerebral cortex. Am J Ment Defic 88:488-496.

Insel TR, Miller LP, Gelhard RE (1990) The ontogeny of excitatory amino acid receptors in rat forebrain--I. N-methyl-D-aspartate and quisqualate receptors. Neuroscience 35:31-43.

Izquierdo A, Belcher AM, Scott L, Cazares VA, Chen J, O'Dell SJ, Malvaez M, Wu T, Marshall JF (2010) Reversal-specific learning impairments after a binge regimen of methamphetamine in rats: possible involvement of striatal dopamine. Neuropsychopharmacology 35:505-514.

Jang J, Ha HJ, Kim YB, Chung YK, Jung MW (2007) Effects of methamphetamine on single unit activity in rat medial prefrontal cortex in vivo. Neural Plast 2007:29821.

Jentsch JD, Taylor JR (1999) Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. Psychopharmacology (Berl) 146:373-390.

Jones S, Bonci A (2005) Synaptic plasticity and drug addiction. Curr Opin Pharmacol 5:20-25.

Judson R, Langdon SW (2009) Illicit use of prescription stimulants among college students: prescription status, motives, theory of planned behaviour, knowledge and self-diagnostic tendencies. Psychol Health Med 14:97-104.

Jung MW, Qin Y, McNaughton BL, Barnes CA (1998) Firing characteristics of deep layer neurons in prefrontal cortex in rats performing spatial working memory tasks. Cereb Cortex 8:437-450.

Kalivas PW (2007) Cocaine and amphetamine-like psychostimulants: Neurocircuitry and glutamate neuroplasticity. Dialogues Clin Neurosci 9:389-397.

Kalivas PW, Pierce RC, Cornish J, Sorg BA (1998) A role for sensitization in craving and relapse in cocaine addiction. J Psychopharmacol 12:49-53.

Kalivas PW, Volkow N, Seamans J (2005) Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. Neuron 45:647-650.

Kalivas PW, Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 162:1403-1413.

Kalivas PW, Volkow ND (2011) New medications for drug addiction hiding in glutamatergic neuroplasticity. Mol Psychiatry 16:974-986.

Kalivas PW, O'Brien C (2008) Drug addiction as a pathology of staged neuroplasticity. Neuropsychopharmacology 33:166-180. Kalsbeek A, Voorn P, Buijs RM, Pool CW, Uylings HB (1988) Development of the dopaminergic innervation in the prefrontal cortex of the rat. J Comp Neurol 269:58-72.

Kauer JA, Malenka RC (2007) Synaptic plasticity and addiction. Nat Rev Neurosci 8:844-858.

Khantzian EJ (1985) The self-medication hypothesis of addictive disorders: focus on heroin and cocaine dependence. Am J Psychiatry 142:1259-1264.

Kim HG, Connors BW (1993) Apical dendrites of the neocortex: correlation between sodium- and calcium-dependent spiking and pyramidal cell morphology. J Neurosci 13:5301-5311.

Kirby KN, Petry NM (2004) Heroin and cocaine abusers have higher discount rates for delayed rewards than alcoholics or non-drug-using controls. Addiction 99:461-471.

Klawans HL, Margolin DI (1975) Amphetamine-induced dopaminergic hypersensitivity in guinea pigs. Implications in psychosis and human movement disorders. Arch Gen Psychiatry 32:725-732.

Klein DA, Gulley JM (2009) Reduced sensitivity to the locomotor-stimulant effects of cocaine is associated with increased sensitivity to its discriminative stimulus properties. Behav Pharmacol 20:67-77.

Koob GF (1992) Neural mechanisms of drug reinforcement. Ann N Y Acad Sci 654:171-191.

Koob GF, Volkow ND (2010) Neurocircuitry of addiction. Neuropsychopharmacology 35:217-238.

Kolb B (1990) Animal models for human PFC-related disorders. Prog Brain Res 85:501-519.

Kolb B (1984) Functions of the frontal cortex of the rat: a comparative review. Brain Res 320:65-98.

Kolb B, Gorny G, Li Y, Samaha AN, Robinson TE (2003) Amphetamine or cocaine limits the ability of later experience to promote structural plasticity in the neocortex and nucleus accumbens. Proc Natl Acad Sci U S A 100:10523-10528.

Kolta MG, Shreve P, De Souza V, Uretsky NJ (1985) Time course of the development of the enhanced behavioral and biochemical responses to amphetamine after pretreatment with amphetamine. Neuropharmacology 24:823-829.

Kourrich S, Rothwell PE, Klug JR, Thomas MJ (2007) Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. J Neurosci 27:7921-7928.

Kreek MJ, Nielsen DA, Butelman ER, LaForge KS (2005) Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. Nat Neurosci 8:1450-1457.

Kuczenski R, Everall IP, Crews L, Adame A, Grant I, Masliah E (2007) Escalating dose-multiple binge methamphetamine exposure results in degeneration of the neocortex and limbic system in the rat. Exp Neurol 207:42-51.

Lambert NM, McLeod M, Schenk S (2006) Subjective responses to initial experience with cocaine: an exploration of the incentive-sensitization theory of drug abuse. Addiction 101:713-725.

Laviola G, Macri S, Morley-Fletcher S, Adriani W (2003) Risk-taking behavior in adolescent mice: psychobiological determinants and early epigenetic influence. Neurosci Biobehav Rev 27:19-31.

Laviola G, Adriani W, Terranova ML, Gerra G (1999) Psychobiological risk factors for vulnerability to psychostimulants in human adolescents and animal models. Neurosci Biobehav Rev 23:993-1010.

Laviola G, Wood RD, Kuhn C, Francis R, Spear LP (1995) Cocaine sensitization in periadolescent and adult rats. J Pharmacol Exp Ther 275:345-357.

Lejuez CW, Bornovalova MA, Reynolds EK, Daughters SB, Curtin JJ (2007) Risk factors in the relationship between gender and crack/cocaine. Exp Clin Psychopharmacol 15:165-175.

Leslie CA, Robertson MW, Cutler AJ, Bennett JP,Jr (1991) Postnatal development of D1 dopamine receptors in the medial prefrontal cortex, striatum and nucleus accumbens of normal and neonatal 6-hydroxydopamine treated rats: a quantitative autoradiographic analysis. Brain Res Dev Brain Res 62:109-114.

Levin FR, Kleber HD (1995) Attention-deficit hyperactivity disorder and substance abuse: relationships and implications for treatment. Harv Rev Psychiatry 2: 246-258.

Li Y, Hu XT, Berney TG, Vartanian AJ, Stine CD, Wolf ME, White FJ (1999) Both glutamate receptor antagonists and prefrontal cortex lesions prevent induction of cocaine sensitization and associated neuroadaptations. Synapse 34:169-180.

Lindvall O, Bjorklund A, Divac I (1978) Organization of catecholamine neurons projecting to the frontal cortex in the rat. Brain Res 142:1-24.

Lisman JE (1997) Bursts as a unit of neural information: making unreliable synapses reliable. Trends Neurosci 20:38-43.

Liu S, Cunningham KA (2006) Serotonin2C receptors (5-HT2C R) control expression of cocaine-induced conditioned hyperactivity. Drug Alcohol Depend 81:275-282.

Liu S, Heitz RP, Sampson AR, Zhang W, Bradberry CW (2008) Evidence of temporal cortical dysfunction in rhesus monkeys following chronic cocaine self-administration. Cereb Cortex 18:2109-2116.

Liu X, Matochik JA, Cadet JL, London ED (1998) Smaller volume of prefrontal lobe in polysubstance abusers: a magnetic resonance imaging study. Neuropsychopharmacology 18:243-252.

Lu W, Wolf ME (1999) Repeated amphetamine administration alters AMPA receptor subunit expression in rat nucleus accumbens and medial prefrontal cortex. Synapse 32:119-131.

Lyvers M (2000) "Loss of control" in alcoholism and drug addiction: a neuroscientific interpretation. Exp Clin Psychopharmacol 8:225-249.

Mainen ZF, Sejnowski TJ (1996) Influence of dendritic structure on firing pattern in model neocortical neurons. Nature 382:363-366.

Mair RD, Kauer JA (2007) Amphetamine depresses excitatory synaptic transmission at prefrontal cortical layer V synapses. Neuropharmacology 52:193-199.

Mandt BH, Zahniser NR (2010) Low and high cocaine locomotor responding male Sprague-Dawley rats differ in rapid cocaine-induced regulation of striatal dopamine transporter function. Neuropharmacology 58:605-612.

Mandt BH, Schenk S, Zahniser NR, Allen RM (2008) Individual differences in cocaine-induced locomotor activity in male Sprague-Dawley rats and their acquisition of and motivation to self-administer cocaine. Psychopharmacology (Berl) 201:195-202.

Markham JA, Morris JR, Juraska JM (2007) Neuron number decreases in the rat ventral, but not dorsal, medial prefrontal cortex between adolescence and adulthood. Neuroscience 144:961-968.

Mathews IZ, McCormick CM (2007) Female and male rats in late adolescence differ from adults in amphetamine-induced locomotor activity, but not in conditioned place preference for amphetamine. Behav Pharmacol 18:641-650.

McFarland K, Lapish CC, Kalivas PW (2003) Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 23:3531-3537.

McKetin R, Mattick RP (1998) Attention and memory in illicit amphetamine users: comparison with non-drug-using controls. Drug Alcohol Depend 50:181-184.

Mobini S, Chiang TJ, Ho MY, Bradshaw CM, Szabadi E (2000) Effects of central 5-hydroxytryptamine depletion on sensitivity to delayed and probabilistic reinforcement. Psychopharmacology (Berl) 152:390-397.

Moeller FG, Dougherty DM, Barratt ES, Oderinde V, Mathias CW, Harper RA, Swann AC (2002) Increased impulsivity in cocaine dependent subjects independent of antisocial personality disorder and aggression. Drug Alcohol Depend 68:105-111.

Monterosso J, Ainslie G (1999) Beyond discounting: possible experimental models of impulse control. Psychopharmacology (Berl) 146:339-347.

Morshedi MM, Rademacher DJ, Meredith GE (2009) Increased synapses in the medial prefrontal cortex are associated with repeated amphetamine administration. Synapse 63:126-135.

Muhammad A, Carroll C, Kolb B (2012) Stress during development alters dendritic morphology in the nucleus accumbens and prefrontal cortex. Neuroscience 216:103-109.

Mulder AB, Nordquist RE, Orgut O, Pennartz CM (2003) Learning-related changes in response patterns of prefrontal neurons during instrumental conditioning. Behav Brain Res 146:77-88.

Muller CP, Carey RJ, Salloum JB, Huston JP (2003) Serotonin1A-receptor agonism attenuates the cocaine-induced increase in serotonin levels in the hippocampus and nucleus accumbens but potentiates hyperlocomotion: an in vivo microdialysis study. Neuropharmacology 44:592-603.

Mychasiuk R, Gibb R, Kolb B (2012) Prenatal stress alters dendritic morphology and synaptic connectivity in the prefrontal cortex and hippocampus of developing offspring. Synapse 66:308-314.

Navarra R, Graf R, Huang Y, Logue S, Comery T, Hughes Z, Day M (2008) Effects of atomoxetine and methylphenidate on attention and impulsivity in the 5-choice serial reaction time test. Prog Neuropsychopharmacol Biol Psychiatry 32:34-41.

Nelson AM, Larson GA, Zahniser NR (2009) Low or high cocaine responding rats differ in striatal extracellular dopamine levels and dopamine transporter number. J Pharmacol Exp Ther 331:985-997.

Nogueira L, Kalivas PW, Lavin A (2006) Long-term neuroadaptations produced by withdrawal from repeated cocaine treatment: role of dopaminergic receptors in modulating cortical excitability. J Neurosci 26:12308-12313.

O'Dell SJ, Marshall JF (2000) Repeated administration of methamphetamine damages cells in the somatosensory cortex: overlap with cytochrome oxidase-rich barrels. Synapse 37:32-37.

O'Neil ML, Kuczenski R, Segal DS, Cho AK, Lacan G, Melega WP (2006) Escalating dose pretreatment induces pharmacodynamic and not pharmacokinetic tolerance to a subsequent high-dose methamphetamine binge. Synapse 60:465-473.

Onn SP, Grace AA (2000) Amphetamine withdrawal alters bistable states and cellular coupling in rat prefrontal cortex and nucleus accumbens neurons recorded in vivo. J Neurosci 20:2332-2345.

Ornstein TJ, Iddon JL, Baldacchino AM, Sahakian BJ, London M, Everitt BJ, Robbins TW (2000) Profiles of cognitive dysfunction in chronic amphetamine and heroin abusers. Neuropsychopharmacology 23:113-126.

Paine TA, Dringenberg HC, Olmstead MC (2003) Effects of chronic cocaine on impulsivity: relation to cortical serotonin mechanisms. Behav Brain Res 147:135-147.

Panksepp J (1981) The ontogeny of play in rats. Dev Psychobiol 14:327-332.

Pattij T, Janssen MC, Vanderschuren LJ, Schoffelmeer AN, van Gaalen MM (2007) Involvement of dopamine D1 and D2 receptors in the nucleus accumbens core and shell in inhibitory response control. Psychopharmacology (Berl) 191:587-598.

Paulson PE, Camp DM, Robinson TE (1991) Time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats. Psychopharmacology (Berl) 103:480-492.

Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates. San Diego: Academic.

Peterson JD, Wolf ME, White FJ (2003) Impaired DRL 30 performance during amphetamine withdrawal. Behav Brain Res 143:101-108.

Peterson JD, Wolf ME, White FJ (2006) Repeated amphetamine administration decreases D1 dopamine receptor-mediated inhibition of voltage-gated sodium currents in the prefrontal cortex. J Neurosci 26:3164-3168.

Peterson JD, Wolf ME, White FJ (2000) Altered responsiveness of medial prefrontal cortex neurons to glutamate and dopamine after withdrawal from repeated amphetamine treatment. Synapse 36:342-344.

Pezze MA, Dalley JW, Robbins TW (2007) Differential roles of dopamine D1 and D2 receptors in the nucleus accumbens in attentional performance on the five-choice serial reaction time task. Neuropsychopharmacology 32:273-283.

Pierce RC, Bell K, Duffy P, Kalivas PW (1996) Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. J Neurosci 16:1550-1560.

Poulos CX, Parker JL, Le AD (1996) Dexfenfluramine and 8-OH-DPAT modulate impulsivity in a delay-of-reward paradigm: implications for a correspondence with alcohol consumption. Behav Pharmacol 7:395-399.

Przegalinski E, Filip M (1997) Stimulation of serotonin (5-HT)1A receptors attenuates the locomotor, but not the discriminative, effects of amphetamine and cocaine in rats. Behav Pharmacol 8:699-706.

Ragozzino ME, Detrick S, Kesner RP (1999) Involvement of the prelimbic-infralimbic areas of the rodent prefrontal cortex in behavioral flexibility for place and response learning. J Neurosci 19:4585-4594.

Rapoport JL, Giedd JN, Blumenthal J, Hamburger S, Jeffries N, Fernandez T, Nicolson R, Bedwell J, Lenane M, Zijdenbos A, Paus T, Evans A (1999) Progressive

cortical change during adolescence in childhood-onset schizophrenia. A longitudinal magnetic resonance imaging study. Arch Gen Psychiatry 56:649-654.

Rebec GV, Sun W (2005) Neuronal substrates of relapse to cocaine-seeking behavior: role of prefrontal cortex. J Exp Anal Behav 84:653-666.

Reynolds B (2006) A review of delay-discounting research with humans: relations to drug use and gambling. Behav Pharmacol 17:651-667.

Ricaurte GA, Schuster CR, Seiden LS (1980) Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: a regional study. Brain Res 193:153-163.

Richards JB, Sabol KE, de Wit H (1999) Effects of methamphetamine on the adjusting amount procedure, a model of impulsive behavior in rats. Psychopharmacology (Berl) 146:432-439.

Richetto J, Feldon J, Riva MA, Meyer U (2013) Comparison of the long-term consequences of withdrawal from repeated amphetamine exposure in adolescence and adulthood on information processing and locomotor sensitization in mice. Eur Neuropsychopharmacol 23:160-170.

Richman CL, Dember WN (1986) Spontaneous alternation behaviour in animals: a review. Curr Psychol Res and Rev 5:358-391.

Robbins TW, Everitt BJ (1999) Drug addiction: bad habits add up. Nature 398:567-570.

Robbins TW, Everitt BJ (2002) Limbic-striatal memory systems and drug addiction. Neurobiol Learn Mem 78:625-636.

Robinson TE, Becker JB (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res 396:157-198.

Robinson TE, Becker JB, Moore CJ, Castaneda E, Mittleman G (1985) Enduring enhancement in frontal cortex dopamine utilization in an animal model of amphetamine psychosis. Brain Res 343:374-377.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 18:247-291.

Robinson TE, Berridge KC (2000) The psychology and neurobiology of addiction: an incentive-sensitization view. Addiction 95 Suppl 2:S91-117.

Robinson TE, Gorny G, Mitton E, Kolb B (2001) Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. Synapse 39:257-266.

Robinson TE, Kolb B (2004) Structural plasticity associated with exposure to drugs of abuse. Neuropharmacology 47 Suppl 1:33-46.

Robinson TE, Kolb B (1999) Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. Eur J Neurosci 11:1598-1604.

Robinson TE, Kolb B (1997) Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. J Neurosci 17:8491-8497.

Roesch MR, Takahashi Y, Gugsa N, Bissonette GB, Schoenbaum G (2007) Previous cocaine exposure makes rats hypersensitive to both delay and reward magnitude. J Neurosci 27:245-250.

Rogers RD, Everitt BJ, Baldacchino A, Blackshaw AJ, Swainson R, Wynne K, Baker NB, Hunter J, Carthy T, Booker E, London M, Deakin JF, Sahakian BJ, Robbins TW (1999) Dissociable deficits in the decision-making cognition of chronic amphetamine abusers, opiate abusers, patients with focal damage to prefrontal cortex, and tryptophan-depleted normal volunteers: evidence for monoaminergic mechanisms. Neuropsychopharmacology 20:322-339.

Rosenberg DR, Lewis DA (1994) Changes in the dopaminergic innervation of monkey prefrontal cortex during late postnatal development: a tyrosine hydroxylase immunohistochemical study. Biol Psychiatry 36:272-277.

Rosenzweig MR, Krech D, Bennett EL, Diamond MC (1962) Effects of environmental complexity and training on brain chemistry and anatomy: A replication and extension. J Comp Physiol Psychol 55:429-437.

Rosselli M, Ardila A (1996) Cognitive effects of cocaine and polydrug abuse. J Clin Exp Neuropsychol 18:122-135.

Rotaru DC, Yoshino H, Lewis DA, Ermentrout GB, Gonzalez-Burgos G (2011) Glutamate receptor subtypes mediating synaptic activation of prefrontal cortex neurons: Relevance for schizophrenia. J Neurosci 31:142-156.

Russig H, Durrer A, Yee BK, Murphy CA, Feldon J (2003) The acquisition, retention and reversal of spatial learning in the morris water maze task following withdrawal from an escalating dosage schedule of amphetamine in wistar rats. Neuroscience 119:167-179.

Saal D, Dong Y, Bonci A, Malenka RC (2003) Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. Neuron 37:577-582.

Sabeti J, Gerhardt GA, Zahniser NR (2002) Acute cocaine differentially alters accumbens and striatal dopamine clearance in low and high cocaine locomotor responders: behavioral and electrochemical recordings in freely moving rats. J Pharmacol Exp Ther 302:1201-1211.

Sabeti J, Gerhardt GA, Zahniser NR (2003) Individual differences in cocaine-induced locomotor sensitization in low and high cocaine locomotor-responding rats are associated with differential inhibition of dopamine clearance in nucleus accumbens. J Pharmacol Exp Ther 305:180-190.

Santucci AC, Rabidou D (2011) Residual performance impairments in adult rats trained on an object discrimination task subsequent to cocaine administration during adolescence. Addict Biol 16:30-42.

Schafer J, Brown SA (1991) Marijuana and cocaine effect expectancies and drug use patterns. J Consult Clin Psychol 59:558-565.

Schenk S, Snow S, Horger BA (1991) Pre-exposure to amphetamine but not nicotine sensitizes rats to the motor activating effect of cocaine. Psychopharmacology (Berl) 103:62-66.

Schoenbaum G, Setlow B (2005) Cocaine makes actions insensitive to outcomes but not extinction: implications for altered orbitofrontal-amygdalar function. Cereb Cortex 15:1162-1169.

Schoenbaum G, Setlow B, Ramus SJ (2003) A systems approach to orbitofrontal cortex function: recordings in rat orbitofrontal cortex reveal interactions with different learning systems. Behav Brain Res 146:19-29.

Schoenbaum G, Saddoris MP, Ramus SJ, Shaham Y, Setlow B (2004) Cocaine-experienced rats exhibit learning deficits in a task sensitive to orbitofrontal cortex lesions. Eur J Neurosci 19:1997-2002.

Schroder N, O'Dell SJ, Marshall JF (2003) Neurotoxic methamphetamine regimen severely impairs recognition memory in rats. Synapse 49:89-96.

Seamans JK, Yang CR (2004) The principal features and mechanisms of dopamine modulation in the prefrontal cortex. Prog Neurobiol 74:1-58.

Seamans JK, Floresco SB, Phillips AG (1995) Functional differences between the prelimbic and anterior cingulate regions of the rat prefrontal cortex. Behav Neurosci 109:1063-1073.

Segal DS, Kuczenski R (1997) An escalating dose "binge" model of amphetamine psychosis: Behavioral and neurochemical characteristics. J Neurosci 17:2551-2566.

Segal DS, Mandell AJ (1974) Long-term administration of d-amphetamine: progressive augmentation of motor activity and stereotypy. Pharmacol Biochem Behav 2:249-255.

Segal DS, Schuckit MA (1983) Animal models of stimulant-induced psychosis. In: Stimulants: Neurochemical, behavioral, and clinical perspectives (Creese I, ed), pp131-167. New York: Raven Press.

Segal DS, Kuczenski R, O'Neil ML, Melega WP, Cho AK (2003) Escalating dose methamphetamine pretreatment alters the behavioral and neurochemical profiles associated with exposure to a high-dose methamphetamine binge. Neuropsychopharmacology 28:1730-1740.

Seiden LS, MacPhail RC, Oglesby MW (1975) Catecholamines and drug-behavior interactions. Fed Proc 34:1823-1831.

Seiden LS, Sabol KE, Ricaurte GA (1993) Amphetamine: effects on catecholamine systems and behavior. Annu Rev Pharmacol Toxicol 33:639-677.

Selemon LD, Begovic A, Goldman-Rakic PS, Castner SA (2007) Amphetamine sensitization alters dendritic morphology in prefrontal cortical pyramidal neurons in the non-human primate. Neuropsychopharmacology 32:919-931.

Sesack SR, Deutch AY, Roth RH, Bunney BS (1989) Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. J Comp Neurol 290:213-242.

Shaw P, Eckstrand K, Sharp W, Blumenthal J, Lerch JP, Greenstein D, Clasen L, Evans A, Giedd J, Rapoport JL (2007) Attention-deficit/hyperactivity disorder is characterized by a delay in cortical maturation. Proc Natl Acad Sci U S A 104:19649-19654.

Simon NW, Mendez IA, Setlow B (2007) Cocaine exposure causes long-term increases in impulsive choice. Behav Neurosci 121:543-549.

Singer BF, Tanabe LM, Gorny G, Jake-Matthews C, Li Y, Kolb B, Vezina P (2009) Amphetamine-induced changes in dendritic morphology in rat forebrain correspond to associative drug conditioning rather than nonassociative drug sensitization. Biol Psychiatry 65:835-840.

Solanto MV (2000) Clinical psychopharmacology of AD/HD: implications for animal models. Neurosci Biobehav Rev 24:27-30.

Spear LP (2000) The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 24:417-463.

Spear LP, Brake SC (1983) Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats. Dev Psychobiol 16:83-109.

Stalnaker TA, Roesch MR, Franz TM, Burke KA, Schoenbaum G (2006) Abnormal associative encoding in orbitofrontal neurons in cocaine-experienced rats during decision-making. Eur J Neurosci 24:2643-2653.

Stanis JJ, Burns RM, Sherrill LK, Gulley JM (2008a) Disparate cocaine-induced locomotion as a predictor of choice in rats trained in a delay-discounting task. Drug Alcohol Depend 98(1-2):54-62.

Stanis JJ, Marquez Avila H, White MD, Gulley JM (2008b) Dissociation between long-lasting behavioral sensitization to amphetamine and impulsive choice in rats performing a delay-discounting task. Psychopharmacology (Berl) 199(4):539-548.

Stefani MR, Moghaddam B (2002) Effects of repeated treatment with amphetamine or phencyclidine on working memory in the rat. Behav Brain Res 134:267-274.

Stewart J, Vezina P (1991) Extinction procedures abolish conditioned stimulus control but spare sensitized responding to amphetamine. Behav Pharmacol 2:65-71.

Stoffel EC, Cunningham KA (2008) The relationship between the locomotor response to a novel environment and behavioral disinhibition in rats. Drug Alcohol Depend 92:69-78.

Strakowski SM, Sax KW, Setters MJ, Keck PE,Jr (1996) Enhanced response to repeated d-amphetamine challenge: evidence for behavioral sensitization in humans. Biol Psychiatry 40:872-880.

Strakowski SM, Sax KW, Rosenberg HL, DelBello MP, Adler CM (2001) Human response to repeated low-dose d-amphetamine: evidence for behavioral enhancement and tolerance. Neuropsychopharmacology 25:548-554.

Substance Abuse and Mental Health Services Administration (2006) Results from the 2005 National Survey on Drug Use and Health: national findings. Office of Applied Studies, NSDUH Series H-30, DHHS Publication No. SMA 06-4194. Rockville, MD.

Substance Abuse and Mental Health Services Administration (2011) Results from the 2010 National Survey on Drug Use and Health: summary of national findings. NSDUH Series H-41, HHS Publication No. (SMA) 11-4658. Rockville, MD.

Stuss DT, Levine B, Alexander MP, Hong J, Palumbo C, Hamer L, Murphy KJ, Izukawa D (2000) Wisconsin Card Sorting Test performance in patients with focal frontal and posterior brain damage: effects of lesion location and test structure on separable cognitive processes. Neuropsychologia 38:388-402.

Sun W, Rebec GV (2006) Repeated cocaine self-administration alters processing of cocaine-related information in rat prefrontal cortex. J Neurosci 26:8004-8008.

Tau GZ, Peterson BS (2010) Normal development of brain circuits. Neuropsychopharmacology 35:147-168.

Tenn CC, Fletcher PJ, Kapur S (2003) Amphetamine-sensitized animals show a sensorimotor gating and neurochemical abnormality similar to that of schizophrenia. Schizophr Res 64:103-114.

Tenn CC, Kapur S, Fletcher PJ (2005) Sensitization to amphetamine, but not phencyclidine, disrupts prepulse inhibition and latent inhibition. Psychopharmacology (Berl) 180:366-376.

Teter CJ, McCabe SE, LaGrange K, Cranford JA, Boyd CJ (2006) Illicit use of specific prescription stimulants among college students: prevalence, motives, and routes of administration. Pharmacotherapy 26:1501-1510.

Thomas MJ, Beurrier C, Bonci A, Malenka RC (2001) Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. Nat Neurosci 4:1217-1223.

Trantham H, Szumlinski KK, McFarland K, Kalivas PW, Lavin A (2002) Repeated cocaine administration alters the electrophysiological properties of prefrontal cortical neurons. Neuroscience 113:749-753.

Tzschentke TM, Schmidt WJ (1999) Functional heterogeneity of the rat medial prefrontal cortex: Effects of discrete subarea-specific lesions on drug-induced conditioned place preference and behavioural sensitization. Eur J Neurosci 11:4099-4109.

Ujike H, Sato M (2004) Clinical features of sensitization to methamphetamine observed in patients with methamphetamine dependence and psychosis. Ann N Y Acad Sci 1025:279-287.

UNODC (2012) World Drug Report. United Nations publication, Sales No. E.12.X1.1.

Uslaner JM, Robinson TE (2006) Subthalamic nucleus lesions increase impulsive action and decrease impulsive choice - mediation by enhanced incentive motivation? Eur J Neurosci 24:2345-2354.

van Gaalen MM, van Koten R, Schoffelmeer AN, Vanderschuren LJ (2006) Critical involvement of dopaminergic neurotransmission in impulsive decision making. Biol Psychiatry 60:66-73.

Verdejo-Garcia AJ, Perales JC, Perez-Garcia M (2007) Cognitive impulsivity in cocaine and heroin polysubstance abusers. Addict Behav 32:950-966.

Vertes RP (2002) Analysis of projections from the medial prefrontal cortex to the thalamus in the rat, with emphasis on nucleus reuniens. J Comp Neurol 442:163-187.

Vezina P (1996) D1 dopamine receptor activation is necessary for the induction of sensitization by amphetamine in the ventral tegmental area. J Neurosci 16:2411-2420.

Vijayraghavan S, Wang M, Birnbaum SG, Williams GV, Arnsten AF (2007) Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. Nat Neurosci 10:376-384.

Volkow ND, Chang L, Wang GJ, Fowler JS, Franceschi D, Sedler M, Gatley SJ, Miller E, Hitzemann R, Ding YS, Logan J (2001) Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. J Neurosci 21:9414-9418.

Vorhees CV, Reed TM, Morford LL, Fukumura M, Wood SL, Brown CA, Skelton MR, McCrea AE, Rock SL, Williams MT (2005) Periadolescent rats (P41-50) exhibit increased susceptibility to D-methamphetamine-induced long-term spatial and sequential learning deficits compared to juvenile (P21-30 or P31-40) or adult rats (P51-60). Neurotoxicol Teratol 27:117-134.

Wang M, Yang Y, Wang CJ, Gamo NJ, Jin LE, Mazer JA, Morrison JH, Wang XJ, Arnsten AF (2013) NMDA receptors subserve persistent neuronal firing during working memory in dorsolateral prefrontal cortex. Neuron 77:736-749.

Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ (2004) Epigenetic programming by maternal behavior. Nat Neurosci 7:847-854.

Wilson JM, Levey AI, Bergeron C, Kalasinsky K, Ang L, Peretti F, Adams VI, Smialek J, Anderson WR, Shannak K, Deck J, Niznik HB, Kish SJ (1996) Striatal dopamine, dopamine transporter, and vesicular monoamine transporter in chronic cocaine users. Ann Neurol 40:428-439.

Winstanley CA, Dalley JW, Theobald DE, Robbins TW (2003) Global 5-HT depletion attenuates the ability of amphetamine to decrease impulsive choice on a delay-discounting task in rats. Psychopharmacology (Berl) 170:320-331.

Winstanley CA, Eagle DM, Robbins TW (2006a) Behavioral models of impulsivity in relation to ADHD: translation between clinical and preclinical studies. Clin Psychol Rev 26:379-395.

Winstanley CA, LaPlant Q, Theobald DE, Green TA, Bachtell RK, Perrotti LI, DiLeone RJ, Russo SJ, Garth WJ, Self DW, Nestler EJ (2007) DeltaFosB induction in orbitofrontal cortex mediates tolerance to cocaine-induced cognitive dysfunction. J Neurosci 27:10497-10507

Winstanley CA, Theobald DE, Dalley JW, Cardinal RN, Robbins,TW (2006b) Double dissociation between serotonergic and dopaminergic modulation of medial prefrontal and orbitofrontal cortex during a test of impulsive choice. Cereb Cortex 16:106-114.

Winstanley CA, Theobald DE, Dalley JW, Robbins TW (2005) Interactions between serotonin and dopamine in the control of impulsive choice in rats: therapeutic

implications for impulse control disorders. Neuropsychopharmacology 30:669-682.

Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. Psychol Rev 94:469-492.

Wogar MA, Bradshaw CM, Szabadi E (1993) Effect of lesions of the ascending 5-hydroxytryptaminergic pathways on choice between delayed reinforcers. Psychopharmacology (Berl) 111:239-243.

Wolf ME, Dahlin SL, Hu XT, Xue CJ, White K (1995) Effects of lesions of prefrontal cortex, amygdala, or fornix on behavioral sensitization to amphetamine: comparison with N-methyl-D-aspartate antagonists. Neuroscience 69:417-439.

Wolf ME, Sun X, Mangiavacchi S, Chao SZ (2004) Psychomotor stimulants and neuronal plasticity. Neuropharmacology 47 Suppl 1:61-79.

Yamamoto DJ, Zahniser NR (2012) Differences in rat dorsal striatal NMDA and AMPA receptors following acute and repeated cocaine-induced locomotor activation. PLoS One 7:e37673.

Zahrt J, Taylor JR, Mathew RG, Arnsten AF (1997) Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. J Neurosci 17:8528-8535.

Zhang XH, Liu SS, Yi F, Zhuo M, Li BM (2013) Delay-dependent impairment of spatial working memory with inhibition of NR2B-containing NMDA receptors in hippocampal CA1 region of rats. Mol Brain 6:13-6606-6-13.