

THE EFFECTS OF REPEATED AMPHETAMINE EXPOSURE DURING  
ADOLESCENCE ON BEHAVIOR AND PREFRONTAL CORTEX FUNCTION

BY

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DISSERTATION

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## **Abstract**

Cognitive impairment and altered drug sensitivity are two commonly reported behavioral outcomes of amphetamine abuse. Individuals who begin using amphetamine during adolescence may have an increased risk of developing drug-related problems because of maturational changes in mesocorticolimbic circuitry that are specific to this stage of development. The studies presented here were designed to assess long-term effects of amphetamine on cognition, dopamine receptor function, and prefrontal cortex (PFC) activity, with a focus on the consequences of drug exposure during adolescence. Chapter 1 includes a review of the literature on substance abuse and PFC dysfunction along with the specific aims of the studies described in the following chapters. Chapter 2 describes studies of drug-induced psychomotor activity and measures of working memory in rats exposed to amphetamine during adolescence or adulthood. Results suggest long-term effects of amphetamine on cognition vary according to the age of exposure. The experiments in Chapter 3 investigated the protracted effects of repeated amphetamine exposure during adolescence on psychomotor behavior and medial PFC function in young adulthood. Relative to controls, rats pre-exposed to amphetamine displayed psychomotor sensitization when challenged with amphetamine and heightened responsiveness to D<sub>1</sub> and D<sub>2</sub> receptor agonists. Expression of sensitization to amphetamine was attenuated in pre-exposed rats following challenges with a D<sub>1</sub> or D<sub>2</sub> receptor antagonist. The long-term functional impact of amphetamine on medial PFC neurons was assessed using single-unit recordings in awake behaving rats. Young adult rats were challenged with amphetamine followed by a D<sub>1</sub> or D<sub>2</sub> receptor antagonist. The proportion of

amphetamine-responsive neurons and the pattern of spike activity was altered in animals exposed to amphetamine during adolescence relative to controls. Finally, Chapter 4 includes a general discussion on the results and implications of the experiments described in this dissertation. Taken together, the research presented here demonstrates age-dependent effects of amphetamine on cognition and highlights the long-lasting impact of amphetamine exposure on dopamine and medial PFC function.

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## **Chapter 1. General Introduction**

### **Substance Abuse and Prefrontal Cortex Dysfunction**

According to the United Nations Office on Drugs and Crime, over 200 million people worldwide use illicit substances each year (UNODC, 2011). In the United States alone, illicit drug abuse and substance use disorders cost nearly \$500 billion annually (Koob and Le Moal, 2006). The availability and use of psychoactive substances increased dramatically over the 20<sup>th</sup> century. In 1971, a growing concern over the rampant use of these drugs led President Richard Nixon to declare drug abuse “public enemy number one.” Yet, drug use and abuse continues to be widespread in our society despite boundless legislative and judicial efforts over the last 50 years. In fact, Americans, while only representing 4% of the human population, use approximately two-thirds of the world’s illicit drugs (Manchikanti, 2007). Over 90 million people in the United States currently have a substance use disorder, which accounts for nearly 50% of the total incidence of mental illness in the country (Regier et al., 1988; Kessler et al., 1994; Madras, 2010).

While trends in drug use change over time, amphetamine remains one of the most commonly used illicit drugs in the world – second only to marijuana (UNODC, 2011). In the United States, nearly 20 million people 12 years of age and older report using amphetamine in the previous month and nearly 500,000 Americans currently suffer from amphetamine dependence (Johnston et al., 2012). Nonetheless, amphetamine remains a schedule II substance with well documented clinical efficacy (Advokat, 2010). Effective for the treatment of attention-deficit hyperactivity disorder (ADHD) and narcolepsy, amphetamine is capable of enhancing cognitive performance,

mood, arousal, alertness, and motivation (Weitzner, 1965; Holliday et al., 1964; Smith and Davis, 1977; Chait et al., 1988; Kelly et al., 1993; Soetens et al., 1993, 1995; Gabbay, 2003; Childs and de Wit, 2009, 2011). However, misuse of this drug can lead to a host of physiological and psychological problems (Berman et al., 2009).

Previous reports indicate that people with a history of amphetamine abuse display significant deficits in decision-making and information processing (Rogers et al., 1999; Ornstein et al., 2000). Interestingly, these deficits were found to be more severe in amphetamine users compared to those who use other drugs of abuse (e.g. opiates, Rogers et al., 1999). Recent clinical findings suggest that cognitive impairment is not just an outcome of drug abuse, but may actually contribute to the etiology of substance use disorders. In patients seeking treatment for psychostimulant dependence, the severity of cognitive dysfunction is predictive of relapse and dropout during drug rehabilitation programs (Aharonovich et al., 2006; Vocci, 2008; Fox et al., 2009). Hence, drug users who display the greatest cognitive impairment have the least success in taking control over their drug use.

There is mounting evidence that uncontrollable drug use is due to neuroadaptations within the prefrontal cortex (PFC) and corticolimbic circuitry (Goldstein and Volkow, 2002; Bolla et al., 2004; Kalivas, 2009; Connolly et al., 2011). The cognitive deficits that emerge following prolonged exposure to psychostimulants overlap with those displayed by people with PFC damage (McKetin and Mattick, 1997, 1998; Jentsch and Taylor, 1999; Rogers et al., 1999; Ornstein et al., 2000; van Holst and Schilt, 2011). Cognitive functions that are mediated by the PFC are particularly sensitive to repeated drug exposure (Jentsch and Taylor, 1999; Rogers and Robbins,

2001). For example, individuals characterized as highly dependent on amphetamine are impaired on tests of verbal memory, attention, and delayed recall (McKetin and Mattick, 1997, 1998). Thus, it's plausible that sub-optimal performance on goal-directed tasks involving these cognitive skills may be related to drug-induced plasticity in the PFC.

With extensive connectivity to other regions of the mesocorticolimbic circuit, the medial PFC plays a key role in goal-directed behavior (Robbins, 1996; Hauser, 1999). The PFC subserves a wide variety of cognitive functions, including so-called “higher-order” executive functions, as well as conditioned behaviors. Previous studies indicate that damaging the medial PFC, or disrupting connectivity between the medial PFC and mesolimbic nodes in this circuitry, can lead to impairments in cognitive performance. For example, disconnection of medial PFC input to the nucleus accumbens impedes behavioral flexibility in the attentional set-shifting task (Goto and Grace, 2005). Intact medial PFC functioning is also important for instrumental and Pavlovian conditioning (Baldwin et al., 2002; Chudasama and Robbins, 2003; Hitchcott et al., 2007; Homayoun and Moghaddam, 2009; Naneix et al., 2009; Nelson, et al., 2010; Winter et al., 2009; Lex and Hauber, 2010). Instrumental conditioning is predicated on the ability of an animal to learn and remember the contingencies between their own actions and outcomes. In contingency degradation tests, control rats adjust their behavior (i.e., decrease lever pressing) when food rewards are no longer contingent on lever pressing (i.e., food pellets are delivered randomly). Rats with medial PFC lesions, in contrast, fail to recognize the change in contingency and continue to emit responses at a high rate (Corbit and Balleine, 2003; Naniex et al., 2009). Interestingly, the medial PFC seems to

be particularly important during acquisition of contingencies. Inactivation of this brain region after training does not alter the expression of action-outcome associations already acquired (Tran-Tu-Yen et al., 2009). Lesions of the medial PFC also disrupt animals' ability to encode Pavlovian stimuli and adjust their behavior using the predictive information relayed by conditioned stimuli. Lesioned animals show indiscriminate Pavlovian conditioned responses, while intact animals learn stimulus associations and primarily emit conditioned responses when conditioned stimuli are presented (Broersen et al., 1996; Parkinson et al., 2000; Nelson et al., 2010).

Lesioning the PFC can lead to significant performance deficits on tasks that require animals to allocate attention to and remember various features of reward-related stimuli. Damage to the medial PFC impairs response accuracy in working memory tasks, including the 5-choice serial reaction time task (Muir et al., 1996), attentional set-shifting (Birrell and Brown, 2000; McGaughy et al., 2008), delayed alternation (Izaki et al., 2008), and operant delayed matching-to-position (DMTP) tasks (Sloan et al., 2006). The medial PFC plays a relatively selective and dissociable role in cognition, and damage does not always lead to measurable changes in cognitive performance. For example, inactivation of the medial PFC does not seem to influence spatial learning in the Morris water maze (Sloan et al., 2006). However, there are many behavioral assays that may be used to probe medial PFC function. Among them, the DMTP task is well suited to assess distinct changes in working memory and mnemonic processes (Dunnett, 1985). Optimal performance on DMTP requires animals to remember the location of a sample stimulus (e.g., lever) over short delay intervals that change across trials. Because this task includes discrete stimulus events and trials, the contribution of



attention and memory processes to performance can be dissociated. For example, changes in attentional control can be measured by analyzing responsiveness to the sample stimulus and to changes in choice accuracy that are independent of delay. Working memory function may be assessed by measuring choice accuracy across the different delay intervals.

The effects of psychostimulants on executive function and conditioned behavior have been well documented in both humans (Weitzner et al., 1965; Hurst et al., 1969) and laboratory animals (Stein, 1964; Clark and Steele, 1966). Amphetamine, in particular, produces marked effects on attention, decision-making, working memory, conditioned behavior, and reward-related learning (Clark and Steele, 1966; Wyvell and Berridge, 2001; O'Tuathaigh et al., 2004; Tindell et al., 2005; Horsley et al., 2008; Gerdjikov et al., 2011). While some studies report cognitive improvement with amphetamine (Hitchcott et al., 1997; Harmer and Phillips, 1998; 1999; Simon and Setlow, 2006; Mendez et al, 2009), others argue that these changes in cognition may be pathological in nature and ultimately contribute to maladaptive goal-directed behavior (Taylor and Robbins, 1984; Taylor and Horger, 1999; Wyvell and Berridge, 2001; Everitt and Wolf, 2002; Kantini et al., 2004; Simon et al., 2009). For example, repeated administration of 2 mg/kg amphetamine for five days enhances rats' acquisition of an appetitive associative learning task (Harmer and Phillips, 1998); however, similar treatment regimens have the potential to impair goal-directed behavior by disrupting cognitive executive functioning.

Amphetamine has a significant impact on cognitive processes mediated by the PFC. However, the majority of studies have focused on the short-term effects of

amphetamine, while the long-term cognitive consequences of repeated amphetamine exposure are not well characterized, particularly in populations with heightened sensitivity to psychostimulant-induced plasticity (e.g., adolescents).

## **Summary & Rationale**

Each year, more people abuse amphetamine than heroin and cocaine combined (UNODC, 2011). What's more, cognitive functioning in those who abuse amphetamine is often so disordered that their performance on cognitive assessments cannot be distinguished from people with frontal lobe damage or schizophrenia (Rogers et al., 1999; Miller and Cohen, 2001; DSM-IV-TR, 2000). Findings from clinical studies indicate that people who chronically abuse amphetamine often display significant impairments in attention, inhibitory control, working memory, information processing, and decision-making (McKetin and Mattick, 1997; 1998; Ornstein et al., 2000; Ersche et al., 2006). There is growing evidence that neuroadaptations in the medial PFC and mesocorticolimbic network underlie these deficits (Goldstein and Volkow, 2002; Jentsch and Taylor, 1999; Kalivas and Volkow, 2005). During adolescence, there are unique structural and functional changes in mesocorticolimbic circuitry occurring, which may increase vulnerability to amphetamine-induced dysfunction. Currently, there is a significant gap in our understanding of how repeated drug exposure during maturation impacts cognition and medial PFC function long-term.

In a collaborative effort, our lab and others have sought to address this knowledge gap. Drawing from a wide variety of behavioral neuroscience techniques, our research over recent years has revealed unique and long-lasting effects of

amphetamine on the brain and behavior as animals transition from adolescence to adulthood. My *central hypothesis* is that mesocorticolimbic circuitry during adolescence is particularly vulnerable to drug-induced plasticity, and repeated exposure to amphetamine during adolescence produces lasting effects on medial PFC neural activity and behavior. I will test this central hypothesis with the following specific aims:

Aim 1. To determine the long-term effects of repeated amphetamine exposure, during adolescence or adulthood, on performance in a medial PFC-sensitive working memory task.

The frontal cortex continues to develop through the adolescent time period (Casey et al., 2005; 2008). As animals mature from adolescence to adulthood, the PFC undergoes significant changes in cell number and morphology (Markham et al., 2007; Cunningham et al., 2002). Glutamate and dopamine systems also continue to mature during this stage of development (Crews et al., 2007; Andersen et al., 2000). Together, these ongoing changes in the brain are thought to leave adolescents particularly vulnerable to drug-induced plasticity (Andersen et al., 2002; Smith, 2003). Dopamine modulates medial PFC output and is thought to mediate PFC participation in attentional control and mnemonic processing via D<sub>1</sub> and D<sub>2</sub> receptor signaling (Seamans et al., 2001). Thus, long-lasting plasticity in dopamine signaling may explain the increased vulnerability that adolescent-exposed animals show to protracted drug-induced behavior.

Altered cognitive function in adolescent-exposed animals is associated with neuroadaptations in mesocorticolimbic circuitry that may be particular to this age group

(Bergstrom et al., 2008). For example, repeated exposure to nicotine during adolescence leads to long-lasting deficits in attention and impulse control that are related to altered glutamate and dopamine activity in the medial PFC (Counotte et al., 2009; 2011). While the majority of studies do not assess if behavioral and neurobiological adaptations are unique to adolescence, Counotte and colleagues included an adult comparison group and found that drug-induced plasticity was not evident in adult-exposed rats. In a recent study, heightened plasticity in mesocortical circuitry in adolescent mice was shown to be regulated by dopamine and glutamate transmission, and interestingly inhibition of dopamine D<sub>2</sub> receptors reversed the long-lasting neuroadaptations in adulthood (Mastwal et al., 2014).

Aim 1 of this dissertation addresses this gap in our knowledge by assessing cognitive performance in rats repeatedly exposed to amphetamine following a protracted drug-free period. Persistent changes in cognition were characterized using an operant-based DMTP task, which has been shown to be sensitive to disruptions of medial PFC function. The experiments described in Chapter 2, investigated the effects of repeated amphetamine exposure on working memory performance and attention using DMTP.

Aim 2. To determine if long-lasting neuroadaptations in dopamine receptor function and medial PFC activity are present in adult rats exposed to amphetamine during adolescence.

In a recent study, we found that repeated exposure to amphetamine during adolescence, but not adulthood, impaired rats' performance on a medial PFC-sensitive

working memory task (Sherrill et al., 2013). Notably, the cognitive disruptive effects of amphetamine were specific to animals exposed during adolescence, a time during which dopamine receptors are in flux and the PFC undergoes significant reconfiguration (Spear, 2000). The relationship between mesocorticolimbic dopamine activity and working memory function has been well documented (Murphy et al., 1996; Romanides et al., 1999; Miller and Cohen, 2001). Altered dopamine signaling is a central feature of psychostimulant sensitization (Pierce and Kalivas, 1997; Steketee, 2003) and may underlie the long-lasting effects of amphetamine on cognition and psychomotor behavior in adolescent-exposed animals (Teicher et al., 1995; Bolanos et al., 1998). The experiments outlined in Chapter 3 were designed to investigate long-lasting effects of adolescent amphetamine exposure on psychomotor activity and dopamine receptor function. In addition, the long-term functional impact of amphetamine on medial PFC neurons was assessed using single-unit recordings in awake behaving rats.

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## **Chapter 2. Age-dependent effects of repeated amphetamine exposure on working memory in rats.<sup>1</sup>**

### **Abstract**

Cognitive dysfunction is a hallmark of chronic psychostimulant misuse. Adolescents may have heightened risk of developing drug-induced deficits because their brains are already undergoing widespread changes in anatomy and function as a normal part of development. To address this hypothesis, we performed two sets of experiments where adolescent and young adult rats were pre-exposed to saline or amphetamine (1 or 3 mg/kg) and subsequently tested in a prefrontal cortex (PFC)-sensitive working memory task. A total of ten injections of amphetamine or saline (in control rats) were given every other day over the course of 19 days. After rats reached adulthood (> 90 days old), cognitive performance was assessed using operant-based delayed matching-to-position (DMTP) and delayed nonmatching-to-position (DNMTP) tasks. DNMTP was also assessed following challenges with amphetamine (0.1-1.25 mg/kg), and ketamine (5.0-10 mg/kg). In experiment one, we also measured the locomotor response following the first and tenth pre-exposure to amphetamine and after an amphetamine challenge given at the conclusion of operant testing. Compared to adult-exposed groups, adolescents were less sensitive to the psychomotor effects of amphetamine. However, they were more vulnerable to exposure-induced cognitive

<sup>1</sup> This work was previously published and is reproduced here with permission from the copyright holders. Bibliographic information: Sherrill LK, Stanis JJ, Gulley JM (2013) Age-dependent effects of repeated amphetamine exposure on working memory in rats. Behav Brain Res 242:84–94.

impairments. For example, adolescent-exposed rats displayed delay-dependent deficits in accuracy, increased sensitivity to proactive interference, and required more training to reach criterion. Drug challenges produced deficits in DNMTTP performance, but these were not dependent on pre-exposure group. These studies demonstrate age of exposure-dependent effects of amphetamine on cognition in a PFC-sensitive task, suggesting a heightened sensitivity of adolescents to amphetamine-induced neuroplasticity.

## Introduction

Amphetamines are among the most commonly used and abused psychoactive drugs, with more people taking them each year than heroin and cocaine combined (UNODC, 2011). In the United States alone, nearly 20 million people 12 years of age and older are currently using amphetamine and nearly 500,000 Americans meet criteria for dependence (SAMHSA, 2011). Clinical studies indicate a history of AMPH abuse is associated with significant deficits in attention, decision-making and information processing (Ersche, Clark, London, Robbins, & Sahakian, 2006; McKetin & Mattick, 1997; McKetin & Mattick, 1998; Ornstein et al., 2000; Rogers et al., 1999; van Holst & Schilt, 2011). In fact, cognitive functioning of chronic abusers is often so disordered that their performance during assessments is difficult to distinguish from that of patients with frontal lobe damage (Miller & Cohen, 2001; Rogers et al., 1999).

Corticolimbic brain circuits, including the interconnected prefrontal cortex (PFC), dorsal striatum, nucleus accumbens, and hippocampus, are critically important for normal cognitive functioning (Arnsten & Rubia, 2012; Miller & Cohen, 2001) and accumulating evidence suggests that drug-induced plasticity in these regions plays an important role in psychostimulant-induced cognitive dysfunction (Goldstein & Volkow, 2002; Jentsch & Taylor, 1999; Kalivas & Volkow, 2005; Vanderschuren & Kalivas, 2000). For example, neuroimaging studies in recovering addicts have revealed that the deficits they exhibit in decision-making and memory are associated with functional abnormalities in the striatum and frontal cortex (Bolla et al., 2003; Volkow et al., 2001). Studies in adult laboratory animals suggest that repeated exposure to amphetamine leads to enduring deficits in attention and working memory that are associated with

reductions and elevations in dopamine and glutamate signaling, respectively, in the PFC (Fletcher, Tenn, Rizos, Lovic, & Kapur, 2005; Fletcher, Tenn, Sinyard, Rizos, & Kapur, 2007; Hedou, Feldon, & Heidbreder, 1999; Hedou, Homberg, Feldon, & Heidbreder, 2001; Lu & Wolf, 1999; Peterson, Wolf, & White, 2000).

One factor that might contribute to amphetamine's potential to induce cognitive dysfunction is the age at which exposure occurs. Like most other drugs of abuse, amphetamine use typically starts during adolescence (DeWit, Offord, & Wong, 1997; Johnston, O'Malley, Bachman, & Schulenberg, 2012), which is the transitional period between childhood and adulthood that begins at approximately 12 years of age and extends to the early or mid-twenties (Dahl, 2004; Lenroot & Giedd, 2006). Notably, the frontal cortex continues to develop throughout the adolescent time period (Casey, Galvan, & Hare, 2005; Casey, Getz, & Galvan, 2008), with alterations in cell number and morphology (Cunningham, Bhattacharyya, & Benes, 2002; Markham, Morris, & Juraska, 2007) and increased synaptic pruning (Huttenlocher & Dabholkar, 1997) among the most prominent changes that occur. Glutamate and dopamine systems also continue to mature during this stage of development (Andersen, Thompson, Rutstein, Hostetter, & Teicher, 2000; Crews, He, & Hodge, 2007; Kalsbeek, Voorn, Buijs, Pool, & Uylings, 1988; Moll et al., 2000). For example, dopamine D<sub>1</sub> and D<sub>2</sub> receptor expression in the rodent PFC, nucleus accumbens and dorsal striatum increases significantly during early adolescence and subsequently declines by as much as 60% as animals reach young adulthood (Andersen et al., 2000; Brenhouse, Sonntag, & Andersen, 2008). Thus, the adolescent brain may be particularly susceptible to drug-induced neuroadaptations and associated cognitive changes because of the unique

effects of drugs in the developing brain (Andersen, Arvanitogiannis, Pliakas, LeBlanc, & Carlezon, 2002; Smith, 2003).

Evidence for enhanced vulnerability to drug-induced plasticity in adolescents has come primarily from studies in rats, where adolescence has been conservatively defined as beginning around postnatal day (P) 28 and extending to P42 (Spear, 2000) or perhaps as late as P60 (Brenhouse & Andersen, 2011; Tirelli, Laviola, & Adriani, 2003). For example, studies of adults exposed to amphetamine, cocaine, methylphenidate, or nicotine during adolescence have reported enduring deficits in cognitive tasks that assess attention, memory, decision making, and impulse control (Adriani et al., 2006; Bethancourt, Camarena, & Britton, 2009; Counotte et al., 2009; Hankosky & Gulley, 2012; Harvey, Dembro, Rajagopalan, Mutebi, & Kantak, 2009; Richetto, Feldon, Riva, & Meyer, 2012; Santucci & Rabidou, 2011; Vorhees et al., 2005). Some of these cognitive dysfunctions have been associated with alterations in multiple measures of neural function and gene expression in the PFC, dorsal striatum and nucleus accumbens (Adriani et al., 2006; Adriani, Canese, Podo, & Laviola, 2007; Black et al., 2006; Counotte et al., 2009; Counotte et al., 2011; Harvey et al., 2009; Labonte et al., 2011). In the majority of these studies, however, it is difficult to ascertain if adolescents are relatively more sensitive to these effects of drug exposure because comparison groups of adult-exposed subjects were rarely utilized.

The primary aim of the current study was to investigate long-lasting effects of repeated amphetamine exposure on locomotor sensitization and cognition in groups of subjects exposed during adolescence or adulthood. In Experiment 1, locomotor activity was measured in an open-field arena after the first and last exposure injection and

following cognitive testing. Working memory was assessed using an operant-based delayed matching-to-position (DMTP) task that is sensitive to disruptions in medial PFC function (Chudasama & Muir, 1997; Sloan, Good, & Dunnett, 2006). After rats learned the task, we also assessed reversal learning by switching to a delayed nonmatching-to-position (DNMTP) task and then subsequently evaluated the effects of pharmacological challenges on task performance using amphetamine and the NMDA antagonist ketamine. In Experiment 2, procedural modifications were made to minimize differences in injection and rearing experiences across groups and to increase the difficulty of the working memory component of the task.

## **Materials and Methods**

Subjects. The male subjects used in these experiments were offspring of male and female Sprague-Dawley rats that were originally obtained from Harlan (Indianapolis, IN, USA) and were bred in our animal facility. The exception to this was the adult-exposed groups used in Experiment 1; these rats were obtained from Harlan, shipped to our facility when they were postnatal day (P) 75, and housed individually upon their arrival. Rats born in our facility were housed 2-3 per cage following weaning at P24 and were housed individually after P85. All rats were maintained on a 12:12 hr light/dark cycle (lights on at 0800) with experimental sessions conducted between 0900 and 1800 hr. Rats were handled at least three times for  $\geq 15$  min each prior to being used in experiments. Food was available *ad libitum* before rats were housed individually, but was restricted during operant training and testing so that rats' weights were maintained at approximately 85% of their free feeding weight. Water was always



available *ad libitum*. Experimental procedures were approved by the Institutional Animal Care and Use Committee 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 monitored in open-field arenas (41 x 41 x 41 cm) consisting of transparent acrylic walls and surrounded by photobeam frames (Coulbourn Instruments; Allentown, PA, USA) that recorded horizontal (lower frame; 2.5 cm above the arena floor) and vertical activity (15 cm above the arena floor). Computer software (TruScan v 2.01, Coulbourn Instruments) was used to record photobeam breaks and to calculate distance traveled (m). Each open-field arena was housed in a sound-attenuating cubicle (76 x 80 x 63 cm) that contained a 76 mm speaker fixed to one side wall that played white noise (70 dB), two ceiling mounted white lights (4 W each), and a centrally mounted overhead camera that captured video for offline analysis of stereotyped behavior.

Operant behavior was assessed in standard operant chambers (Coulbourn Instruments). The front panel of each chamber contained a centrally located food trough flanked on either side by a retractable lever (i.e., levers A and B). White cue lights were mounted above each lever. The rear wall contained a white houselight located near the top of the chamber and a recessed nosepoke port containing a red LED light was located near the floor. Infrared photobeam detectors that were positioned in the food trough and nosepoke port were used to monitor head entries. Graphic State (v3.1; Coulbourn Instruments) was used for automated chamber control and data collection.

Drugs. D-amphetamine sulfate (Experiments 1 and 2) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in sterile saline (0.9% NaCl). Ketamine HCl (Experiment 1) was obtained in a 100 mg/ml injectable solution (Ketaset; Pfizer Animal Health; Fort Dodge, IA, USA) and diluted with sterile saline to the appropriate concentrations for injection. All dosages were calculated based on the weight of the salt and injections were given at a volume of 1 ml/kg.

### *Experiment 1*

Pre-treatment. The male rats used in this experiment ( $n = 50$ ) were previously used in a study of cocaine-induced locomotor activity and were therefore exposed to a single injection of 10 mg/kg cocaine at either P35 or P95. Subsequently, they were randomly assigned to one of four groups that received 0.9% saline or 3 mg/kg AMPH during late adolescence or young adulthood. Injections were given using an intermittent pattern of exposure with one injection (i.p.) occurring every other day for a total of 10 injections. We previously used this procedure to induce long-lasting behavioral sensitization ( $> 3$  months) in rats exposed in adulthood (Stanis, Marquez Avila, White, & Gulley, 2008). Rats in the adolescent-exposed groups ( $n = 9$  given saline;  $n = 15$  given AMPH) received injections between P37 and P55, whereas those in the adult-exposed group ( $n = 7$  given saline;  $n = 19$  given AMPH) were injected between P98 and P116. For the first and tenth injections, activity was monitored in the open-field arena 30 min before and 60 min after injection. For injections 2-9, rats were injected in a separate test room and were then placed for 60 min in an acrylic tub (46 x 25 x 22 cm) lined with hardwood bedding.

Working memory task. When rats were P85 (adolescent exposed) or P120 (adult exposed), they were placed on food restriction (~15 g/day) and were trained starting 5 days later to respond on one of two levers on a continuous reinforcement schedule. Using procedures adapted from Dunnett (1985), rats were then trained in daily sessions to perform a no-delay version of a matching-to-position task. For these sessions, which consisted of 100 trials, an individual trial began with the illumination of the houselight and a 5-s ITI. Lever A or B was then presented randomly (with equal probability) and rats were required to respond on the extended lever within 10 s (i.e., sample phase). These responses were followed by retraction of this sample lever and illumination of the cue light in the trough. After the rat poked its nose into the trough, the sample lever was presented again (i.e., choice phase) and a food pellet was subsequently delivered after the rat made another response on that lever. If no response was made, the lever was retracted, the houselight was extinguished, and the trial was scored as an omission. After at least two of these training sessions, the procedure was repeated except that both levers were presented during the choice phase. At this training stage, responses on the sample lever were reinforced with food pellet delivery, whereas those that were made on the non-sample lever were scored as incorrect and a 5-s ITI was initiated. Daily training sessions of 112 trials continued until rats achieved  $\geq 85\%$  correct performance on two consecutive sessions.

During the next training stage, trials were modified such that a delay phase was introduced between the sample and choice phases. Delay intervals were randomly selected from one of seven durations, with initial training utilizing “short delays” of 0, 1, 2, 3, 4, 5, and 6 s. After rats met the performance criterion ( $\geq 85\%$  correct choices over

two consecutive sessions), “moderate” delays of 0, 1, 2, 4, 8, 12, and 16 s were introduced. Following initial training, DMTP “long” delays of 0, 2, 4, 8, 12, 18, and 24 s were introduced. This training progression was chosen to avoid response extinction, which sometimes occurs if longer delays are introduced before task acquisition has progressed sufficiently. During these training sessions, each delay was presented on 16 trials, for a total of 112 trials/session. After rats reached criterion at the long delays on DMTP, they were given five additional training sessions (“overtraining”) before the task rule was reversed. In these delayed nonmatching-to-position (DNMTP) sessions, which utilized the long delays (0-24 s), responses during the choice phase that were made on the non-sample lever were reinforced.

Drug challenges. Following the last DNMTP training session (i.e., second consecutive session of  $\geq 85\%$  correct), the effects of challenge injections with AMPH and ketamine were assessed in two testing blocks. For the first block, rats were given injections of vehicle (saline; 1 ml/kg, i.p.) or amphetamine (0.3, 0.75, and 1.25 mg/kg, i.p.) 5 min before they were placed in the operant chamber for a DNMTP test session. Injections were given over five 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 from testing before starting the second block of injections. For these tests, saline vehicle or ketamine (5, 7.5, 10 mg/kg, i.p.) was given 10 min before rats were placed in the chamber for their test session. Injection order was assigned randomly and given over five sessions (SDDDS). After their final operant session, rats were given access to food *ad libitum*. One week later, all rats were challenged with 3 mg/kg amphetamine (i.p.)

using the same open-field locomotor test procedures that were used during their first and tenth pre-treatment injections.

Data analysis. Data from rats pre-treated with saline during late adolescence ( $n = 9$ ) or adulthood ( $n = 7$ ) were combined because there were no statistically significant differences between these groups in measures of open-field behavior and working memory task performance. Locomotor activity (ambulation) was quantified as the total horizontal distance (m) during the first 60 min post-injection. This measure was calculated from consecutive photobeam breaks (i.e., coordinate changes) using computer software (TruScan; Coulbourn Instruments) that subtracted repetitive behaviors occurring in the absence of ambulation. To characterize bouts of repetitive movement (i.e., stereotypy), video recordings of open-field test sessions were scored using a semi-quantitative method we used previously (Stanis et al., 2008). Trained observers that were not given information about a rat's group membership scored 30 s segments of video taken every 5 min of the 60-min post-injection period. For each segment, behavior was rated for intensity (1-mild, 2-intense, or 3-intense) and duration (seconds spent exhibiting the scored behavior; 0-30 s); these values were then multiplied to give a single score that could range from 0-90. These data were analyzed using a two-way ANOVA, with treatment day as within-subjects factors and group as between-subjects factors. All main effects and interactions were further analyzed using one-way ANOVA and Student-Newman-Keuls comparison procedures.

Performance on the working memory task was measured by calculating the mean percentage of correct choices across sessions and delay blocks and the number of sessions to reach criterion. In order to assess delay-dependent changes in

performance during training, rats mean accuracy across delay blocks was compared on the first two sessions a rat from any group met the performance criterion during DMTP (sessions 1 and 2) and DNMTTP (sessions 6 and 7). Separate two-way repeated measures ANOVAs (exposure group x delay) were used to assess delay-dependent changes in performance. A two-way repeated measures ANOVA with treatment group as the between-subjects factor and training phase (DMTP and DNMTTP) as the within-subjects factor was used to analyze the number of sessions to reach criteria. To assess the effects of proactive interference on performance, accuracy was compared across delays on trials in which the choice response made on the previous trial differed from the correct response required on the current trial (i.e. different trials) and on trials where the correct choice and previous choice response were congruent (i.e. same trials). These data were analyzed separately for DMTP and DNMTTP using three-way repeated measures ANOVAs (exposure group x delay x trial type).

Performance following amphetamine and ketamine drug challenges was assessed by calculating three measures: mean percent correct, latency to choice, and the number of trials omitted during each test session. These measures were analyzed using separate two-way repeated measures ANOVAs with exposure group as the between-subjects factor and dose as the within-subjects factor. Proactive interference during drug challenges was assessed using separate three-way ANOVAs (exposure group x trial type x dose) for amphetamine and ketamine. Sessions during which an animal failed to complete > 75% of trials were not included in the analysis of percent correct. All main effects and interactions were further analyzed using one-way ANOVA and Student-Newman-Keuls comparison procedures.

## *Experiment 2*

Pre-treatment. The experimentally naïve, male rats ( $n = 46$ ) used in this experiment were administered saline or amphetamine using similar methods as those used in Experiment 1, but with several changes to the experimental design. First, all rats were offspring of breeders maintained in our facility and they were assigned to exposure groups so that rats from each litter were represented within each group. Second, rats were given injections (i.p.) every other day during both adolescence (P27-45) and young adulthood (P85-103). Those assigned to the control group were given saline (1 ml/kg) at both time points, those in the adolescent-exposed groups were given amphetamine (1 or 3 mg/kg) during adolescence and saline during adulthood, and those in the adult-exposed groups were given saline during adolescence and amphetamine (1 or 3 mg/kg) during adulthood. Following each injection, rats were placed individually into the same type of enclosures that were used during injections 2-9 in Experiment 1, where they remained undisturbed for 60 min post-injection.

Working memory task. Rats began operant training after reaching P120. The animals were food deprived (~85%) over a period of 5 days then began lever press training on a continuous reinforcement schedule. Training on the working memory task was similar to that described in Experiment 1, with the following changes. During each trial, a cue light was illuminated above the corresponding sample lever and three lever presses (FR 3) were required during the sample phase to initiate the delay interval. In addition, during the delay interval, rats were required to nosepoke into the nosepoke port located on the rear wall of the chamber. These modifications were implemented to increase the salience of the sample and to discourage further the development of non-

mnemonic (e.g., positional) strategies (Paule et al., 1998). A final procedural difference from Experiment 1 involved the introduction of longer delay intervals. Rats were trained on DMTP until delay blocks ranged from 0-30 s [delay blocks: 0, 2, 4, 8, 12, 18, 24, 30 s]. Rats progressed to DNMTP (0-30 s delays) once they achieved  $\geq 85\%$  correct on two consecutive sessions.

Data analysis. Performance during training on DMTP and DNMTP was assessed as described for Experiment 1, with individual rats' mean performance across sessions 1 and 2 (DMTP) and 4 and 5 (DNMTP) used in the analysis. Separate two-way ANOVAs for DMTP and DNMTP were conducted to investigate within session delay-dependent changes in accuracy. A two-way repeated measures ANOVA was used to analyze the number of sessions to criterion during DMTP and DNMTP training. Proactive interference during DMTP and DNMTP training was assessed using two- and three-way repeated measures ANOVAs. Sessions during which an animal failed to complete  $> 75\%$  of trials were not included in the analysis of percent correct. All main effects and interactions were further analyzed using one-way ANOVA and Student-Newman-Keuls comparison procedures. All data are presented as group mean  $\pm$  SEM.

## **Results**

### *Experiment 1*

Amphetamine produced significant changes in ambulation and stereotyped behavior following the first (T1) and tenth (T10) injections (Fig. 2.1). These changes in activity were also evident when an amphetamine challenge was given at the conclusion of operant testing, which occurred approximately 4 months after pre-treatment for rats in the adolescent-exposure group and approximately 3 months for those in the adult-



exposed group. Separate two-way ANOVAs revealed significant interactions between group and treatment day for ambulation [ $F(4,92) = 54.7, p < 0.001$ ] and stereotypy [ $F(4,92) = 14.1, p < 0.001$ ]. Compared to saline-treated controls, rats in both the adolescent- and adult-exposed groups exhibited significant increases in ambulation and stereotypy after their first injection with 3 mg/kg amphetamine. For adolescent-exposed rats, ambulation was elevated to a similar magnitude following the tenth injection. In adult-exposed rats, however, there was a significant reduction in ambulation following injection 10 compared to injection 1. Both pre-exposed groups showed an increase in stereotypy at injection 10 and amphetamine challenge compared to controls. In addition, stereotypy in both pre-exposed groups reached a maximal level following the amphetamine challenge injection. Yet, the overall magnitude of the stereotypy response was lower in adolescent-exposed rats compared to the adult-exposed group after the tenth and challenge injections. Thus, sensitization to amphetamine-induced stereotypy was still evident in both pre-exposure groups at the time of amphetamine challenge, and these high levels of stereotypy were associated with a concomitant decrease in ambulatory activity these rats.

Although the changes in amphetamine-induced activity were greatest in rats exposed to the drug during adulthood, significant impairments in performance during the working memory task were more robust in adolescent-exposed rats (Fig. 2.2). Two-way repeated measures ANOVA (group x delay) of DMTP accuracy revealed significant main effects of exposure group [ $F(2,47) = 5.30, p < 0.01$ ] and delay [ $F(6,282) = 172, p < 0.001$ ], and a significant group x delay interaction [ $F(12,282) = 1.88, p < 0.05$ ]. Post-hoc analysis indicated that the accuracy of adolescent-exposed rats was significantly

impaired relative to controls and the adult-exposed group when the delay interval exceeded 12 s (Fig. 2.2A). With repeated training, all rats reached the performance criterion. However, those exposed to amphetamine during adolescence required more sessions than rats in the other groups (Fig. 2.2C). When the task was then reversed to DNMT, performance decreased in all groups and there were no apparent delay-dependent differences (Fig. 2.2B). Adolescent exposed rats did require more trials to reach the performance criterion, however (Fig. 2.2C). Two-way repeated measures ANOVA of the sessions to criterion data revealed significant main effects of group [ $F(2,47) = 4.20, p < 0.05$ ] and training phase [ $F(1,47) = 177, p < 0.001$ ]. The interaction between group and training phase was not significant ( $p > 0.05$ ).

In order to assess the extent to which proactive interference contributed to group differences in DMTP performance, accuracy was analyzed on a trial-by-trial basis (Fig. 2.3). Analysis of these data indicated that rats were less accurate on 'different' trials compared to 'same' trials when the delay interval exceeded 8 s [significant trial type x delay interaction:  $F(6,282) = 5.90, p < 0.001$ ]. Furthermore, on different trials, adolescent-exposed rats were significantly less accurate than control and adult-exposed groups at delay intervals 12 s and longer [significant group x delay interaction:  $F(12,282) = 2.53, p < 0.01$ ]. During DNMT training (data not shown), rats were also less accurate on different compared to same trials [main effect of trial type:  $F(1,46) = 35.3, p < 0.001$ ], but there were no group differences in susceptibility to proactive interference.

After rats met the performance criterion on DNMT, they were tested for their response to pre-session challenge injections of amphetamine or ketamine (Fig. 2.4).

Separate two-way repeated measures ANOVAs (exposure group x dose) for amphetamine and ketamine revealed a main effect of dose for AMPH [ $F(3,6) = 51.9$ ,  $p < 0.001$ ]. Accuracy was significantly impaired following each challenge dose of AMPH. However, there were no significant effects of ketamine on accuracy [NS main effect of dose,  $p > 0.05$ ], and neither drug was found to influence any one particular group more than others [NS main effects of group,  $ps > 0.05$ ]. Separate three-way repeated measures ANOVAs were used to analyze proactive interference during sessions following drug challenges (Fig. 2.5). Amphetamine reduced accuracy on both same and different trials, while ketamine impaired performance only on different trials [significant trial type x dose interactions:  $F_s(3,6) = 21.8$ ,  $ps < 0.001$ ]. Changes in accuracy following amphetamine and ketamine were accompanied by significant increases in the mean number of trials omitted and choice latency (Table 2.1). Following challenge with 0.75 and 1.25 mg/kg amphetamine, and all test doses of ketamine, rats showed a significant increase in omissions [main effects of dose:  $F_s(3,6) = 28.6$  and  $18.0$ , respectively,  $ps < 0.001$ ]. There were no significant effects of amphetamine on choice latency; however, ketamine increased choice latency at all doses tested relative to saline [main effect of dose:  $F(3,6) = 11.7$ ,  $p < 0.001$ ].

## *Experiment 2*

In order to extend the findings from Experiment 1, we modified the pre-treatment and working memory task protocols to include a second amphetamine dose, control procedures for injection experience, and additional task demands for DMTP and DNMTTP. As shown in Figure 2.6, accuracy during DMTP decreased as a function of the delay interval. Two-way repeated measures ANOVA (exposure group x delay) revealed

a main effect of group [ $F(4,41) = 3.20, p < 0.05$ ] and delay [ $F(6,246) = 65.7, p < 0.001$ ]. Rats exposed to 3 mg/kg amphetamine during adolescence were significantly impaired relative to rats exposed to the same dose during adulthood. While there were no significant group differences in DNMTTP performance, all rats showed a significant reduction in accuracy across delays [main effect of delay:  $F(6,246) = 39.2, p < 0.001$ ]. In addition, rats exposed to 1 mg/kg amphetamine during adolescence and both adult-exposed groups required significantly more trials to reach criterion on DNMTTP compared to DMTP [group x training phase interaction:  $F(4,246) = 3.00, p < 0.05$ ]. Follow-up analyses of proactive interference effects revealed that during DMTP training, rats were significantly more accurate on same compared to different trials, with rats exposed to 3 mg/kg amphetamine during adolescence particularly susceptible to proactive interference (Fig. 2.7). The adolescent-exposed group was found to perform significantly worse than all other groups. These effects were confirmed with a three-way repeated measures ANOVA with significant main effects of group [ $F(4,41) = 3.30, p < 0.05$ ], trial type [ $F(1,41) = 88.4, p < 0.001$ ], and delay [ $F(6,246) = 64.4, p < 0.001$ ]. Separate analysis on different trials alone, revealed a significant group x delay interaction, with adolescents exposed to 3 mg/kg performing worse than control and adult-exposed groups [ $F(24,246) = 1.59, p < 0.05$ ]. Analysis of proactive interference during DNMTTP (data not shown) indicated that rats were less accurate on 'different' compared to 'same' trials [main effect of trial type:  $F(1,41) = 59.9, p < 0.001$ ], but there were no statistically significant group differences in susceptibility to proactive interference.

## Discussion

The findings of the present study demonstrate long-lasting effects of amphetamine on cognitive performance that are dependent on the developmental time period during which drug exposure occurs. Adult rats that were exposed to amphetamine during adolescence displayed delay-dependent deficits in choice accuracy, they required more sessions to optimize performance and learn task rules, and they were more susceptible to proactive interference, compared to control and adult-exposed groups. Amphetamine-induced locomotor sensitization, however, was enhanced in adult- compared to adolescent-exposed rats. Thus, amphetamine-induced changes in cognition were dissociable from the drug's lasting effects on sensitivity to its motor activating effects. Moreover, the enhanced vulnerability of adolescents to the disruptive effects of repeated amphetamine exposure in a medial PFC-sensitive cognitive task suggests that these age-dependent effects may be due to amphetamine-induced disruptions in the normal development of the PFC.

Our measures of amphetamine-induced activity in an open-field arena (Experiment 1) revealed similar psychomotor activation in adolescents and adults following an acute injection of 3 mg/kg amphetamine. In addition, after ten intermittent injections of amphetamine, sensitization to the stereotypy-inducing effects of amphetamine were evident in both age groups, but this effect was greater in adult-exposed rats, who also displayed a concomitant reduction in ambulation. A similar pattern of ambulation was observed in both age groups following amphetamine challenge, although adult-exposed rats again showed more robust stereotypy than animals exposed to amphetamine during adolescence. While it is possible that the

additional injections of amphetamine (0.3, 0.75 and 1.25 mg/kg) and ketamine (5, 7.5, and 10 mg/kg) rats received during DNMT1P testing may have influenced the expression of their sensitized behavior following amphetamine challenge, all groups (including controls) had this same experience. Inspection of the data shown in Fig. 1 reveals that the magnitude of amphetamine-induced activity in rats pre-exposed to saline (i.e., controls) was similar to that seen in adult-exposed rats during their first treatment. Thus, it's unlikely that the drug challenges during the working memory task had differential effects among the groups.

Age-dependent differences amphetamine-induced activity in rats and mice have been documented previously, with some studies showing that adults are more sensitive to acute amphetamine compared to adolescents (Mathews & McCormick, 2007; Mathews, Waters, & McCormick, 2009; Zombeck, Gupta, & Rhodes, 2009). Others, however, report no age-dependent differences (Adriani, Chiarotti, & Laviola, 1998; Mathews & McCormick, 2007; Niculescu, Ehrlich, & Unterwald, 2005; Walker et al., 2010). There are also inconsistent findings for amphetamine-induced sensitization. Some studies report greater amphetamine-induced sensitization in adolescent-exposed rodents (Adriani et al., 1998; Kameda et al., 2011; Mathews, Morrissey, & McCormick, 2010; Mathews, Kelly, & McCormick, 2011), whereas others indicate greater effects in adults (Good & Radcliffe, 2011; Richetto, Feldon, Riva, & Meyer, 2012; Zakharova, Leoni, Kichko, & Izenwasser, 2009) or no difference between age groups (Niculescu et al., 2005; Good & Radcliffe, 2011). Methodological differences contribute to some of these discrepant findings, with key factors being amphetamine dose and the aspect of drug-induced behavior that is measured (e.g., locomotion or stereotypy). At lower

doses ( $< 1.5$  mg/kg), adolescents tend to show an attenuated response to the first injection but enhanced locomotor sensitization relative to adults (Bolanos, Glatt, & Jackson, 1998; Mathews & McCormick, 2007; Mathews et al., 2009; Zakharova et al., 2009). With higher doses ( $> 2$  mg/kg), however, age-dependent differences in initial responsiveness diminish and repeated exposure produces robust stereotypy and reduced locomotor activity, particularly in adults, as shown here and elsewhere (Adriani et al., 1998; Adriani & Laviola, 2000). Thus, adolescents appear to have a higher threshold for the psychomotor-activating effects of amphetamine, but once activated their response is similar to that seen in adults. In addition, their qualitatively different pattern of sensitization following repeated exposure suggests the neuroadaptations induced by repeated amphetamine exposure may be unique in adolescents relative to adults. Interestingly, age-dependent differences in amphetamine-induced behavior are often not observed unless subjects experience a period of withdrawal. For example, sensitization is expressed following repeated amphetamine exposure during adolescence only when animals are challenged weeks later (Kolta, Scalzo, Ali, & Holson, 1990; McPherson & Lawrence, 2006). Two potential explanations for this phenomenon are that amphetamine-induced neuroadaptations in adolescent-exposed animals are not evident until adulthood, or alternatively, that plasticity in younger animals is enhanced following an extended drug withdrawal period. Dissociating these two hypotheses in rodents may prove difficult given the relative brevity of the adolescent time period. Nevertheless, future studies are needed to elucidate the potential role that drug withdrawal plays in the age-dependent effects of amphetamine on plasticity and behavior.

In both Experiments 1 and 2, we observed age-dependent differences in the effects of amphetamine on working memory. Rats exposed to 3 mg/kg amphetamine during adolescence, but not those exposed during adulthood, showed delay-dependent deficits in choice accuracy during DMTP training. Analyses of proactive interference indicated the impaired performance of adolescent-exposed rats was largely due to decreased accuracy on different trials. On these trials, their accuracy dropped to near chance at longer delays. This floor effect may have contributed to the lack of group differences in overall accuracy at longer delays observed in Experiment 2. Nevertheless, rats exposed to amphetamine during adolescence in both experiments were more susceptible to proactive interference compared to control and adult-exposed animals. Previous studies suggest that enhanced susceptibility to proactive interference reflects difficulties with encoding and organizing stimulus events and behavioral responses across trials. Thus, accuracy is often worse on trials with incongruent stimuli and/or choices compared to previous trials (Dunnett, 1985; Dunnett & Martel, 1990; Edhouse & White, 1988; Paule et al., 1998). In addition to the deficits in choice accuracy found within sessions, adolescent-exposed rats also required a greater number of sessions to reach performance criterion on both DMTP and DNMT. Age-dependent differences in the rate of acquisition were only observed in Experiment 1. In order to further explore the specificity of amphetamine exposure during adolescence and the degree of cognitive impairment from that exposure, Experiment 2 was performed with a few procedural changes.

First, in order to better control for group differences in rearing environment and injection experience, rats in Experiment 2 were offspring of dams bred in our facility and



all animals received injections during both adolescence and adulthood. Stress, particularly early in life, can have significant neurophysiological and behavioral consequences, especially with regards to the PFC and cognitive processes mediated by this brain region (Cerqueira, Mailliet, Almeida, Jay, & Sousa, 2007; Liston et al., 2006; Spear, 2009; Yuen et al., 2012). The pre-exposure injections in Experiment 2 also began earlier in adolescence (P27) and young adulthood (P85) compared to those in Experiment 1 (P37 and P98 for adolescence and young adulthood, respectively). Second, the working memory task was altered such that a wider range of delay intervals was used and rats were required to perform a response at the nosepoke port on the chamber wall opposite to the sample before the choice phase began. The former change was made because delay-dependent deficits in Experiment 1 were found at the longest delay interval tested (i.e. 24 s). The requirement for a nosepoke response at the back wall of the chamber was implemented to further discourage the use of non-mnemonic strategies (e.g., standing near the position of the sample lever during the delay phase), which may develop as task difficulty increases (Paule et al., 1998). Taken together, the results of these experiments suggest that intermittent exposure to a moderately high dose of amphetamine leads to cognitive dysfunction that is long-lasting and dependent on the age of exposure.

Interestingly, the deficits displayed by adolescent-exposed animals in the present study are similar to those observed in PFC-lesioned animals performing DMTP (Sloan et al., 2006). There are numerous reports showing that damage to the medial PFC produces selective deficits in working memory performance, increased sensitivity to proactive interference, and impaired attention (Chudasama & Muir, 1997; Floresco,

Block, & Tse, 2008; Granon, Vidal, Thinus-Blanc, Changeux, & Poucet, 1994; Porter & Mair, 1997; Sloan et al., 2006). Adolescent-exposed rats in the present study showed decreased accuracy and greater susceptibility to proactive interference that was most pronounced at delay intervals between 12 to 24 s. Rats were not impaired at short delays, suggesting the deficits found here are due primarily to mnemonic dysfunction, rather than delay-independent disruptions in mediating behavior or attention. However, attention deficits cannot be ruled out entirely because rats' accuracy was susceptible to proactive interference, which requires attention allocated to the sample stimulus within each trial (Harper, Wisniewski, Hunt, & Schenk, 2005; White, 2001). Given that adolescent-exposed rats were impaired on different trials at longer, and not shorter delays, suggests that if non-mnemonic deficits were induced by repeated amphetamine exposure, they were not significant enough to impair performance when task demands were relatively easier at short delays (Chrobak, 2008; Chudasama, 1997)

A candidate mechanism for these effects of amphetamine is altered signaling in the mesocorticolimbic system. Previous studies have found enhanced amphetamine-induced plasticity in subcortical regions such as the hippocampus and striatum in adolescent animals (Ehrlich, Sommer, Canas, & Unterwald, 2002; Featherby, van den Buuse, Lubman, & Lawrence, 2008). In addition, there is recent evidence that the PFC undergoes significant structural and functional plasticity with repeated exposure to amphetamines during this age period (Gramage, Del Olmo, Fole, Martin, & Herradon, 2011; Lee, Kim, Lee, & Jang, 2011; Wong & Stevens, 2012). Indeed, amphetamine produces unique age-dependent effects on glutamate and dopamine activity in the PFC (Good, Liang, Patel, & Radcliffe, 2011; Kindlundh-Hogberg, Blomqvist, Malki, & Schioth,

2008; Mathews et al., 2011), with significant changes in neuron excitability and dopamine release in animals exposed to the drug during adolescence (Gramage et al., 2011; Laviola, Pascucci, & Pieretti, 2001; McPherson & Lawrence, 2006). The importance of glutamate and dopamine systems in the performance of working memory tasks has been well documented (Baron, Wright, & Wenger, 1998; Enomoto & Floresco, 2009; Valentim, Alves, Olsson, & Antunes, 2008). In the present study, pharmacological manipulation of these systems with challenge injections of amphetamine and ketamine had differential effects on DNMT performance. Challenge injections of amphetamine dose-dependently reduced accuracy overall, while both drugs enhanced rats' sensitivity to proactive interference. Consistent with previous reports, rats' performance was impaired on different trials following challenge with drugs that target dopamine and glutamate systems (Chrobak, Hinman, & Sabolek, 2008; Harper et al., 2005). Additionally, the number of trial omissions and choice latencies were increased following drug challenge. These findings suggest that rats may have become disoriented while performing the task and as a result they were more susceptible to proactive interference (Chrobak, 2008; Chudasama, 1997). This hypothesis is further supported by studies showing that the organization of delay mediating behaviors and instrumental actions are disrupted by manipulations of dopamine and glutamate activity in the PFC (Baldwin, Sadeghian, & Kelley, 2002; Naneix, Marchand, Di Scala, Pape, & Coutureau, 2009). Nonetheless, there were no differences in performance between controls and amphetamine-exposed groups following drug challenge. Given that drug challenge occurred following extensive training, the lack of group differences is likely due to "overtraining" in the task. At the

time of drug challenges, all animals were performing with similar accuracy despite the fact that they required, on average, more sessions to reach the 85% correct performance criterion. Previous studies indicate that impairments in working memory and proactive interference following PFC lesions dissipate with training (Gisquet-Verrier & Delatour, 2006; Harrison & Mair, 1996; Koger & Mair, 1994). Our findings are consistent with this notion. Thus, while amphetamine-induced cognitive dysfunction persists over long periods of time, deficits may be overcome with extended training procedures.

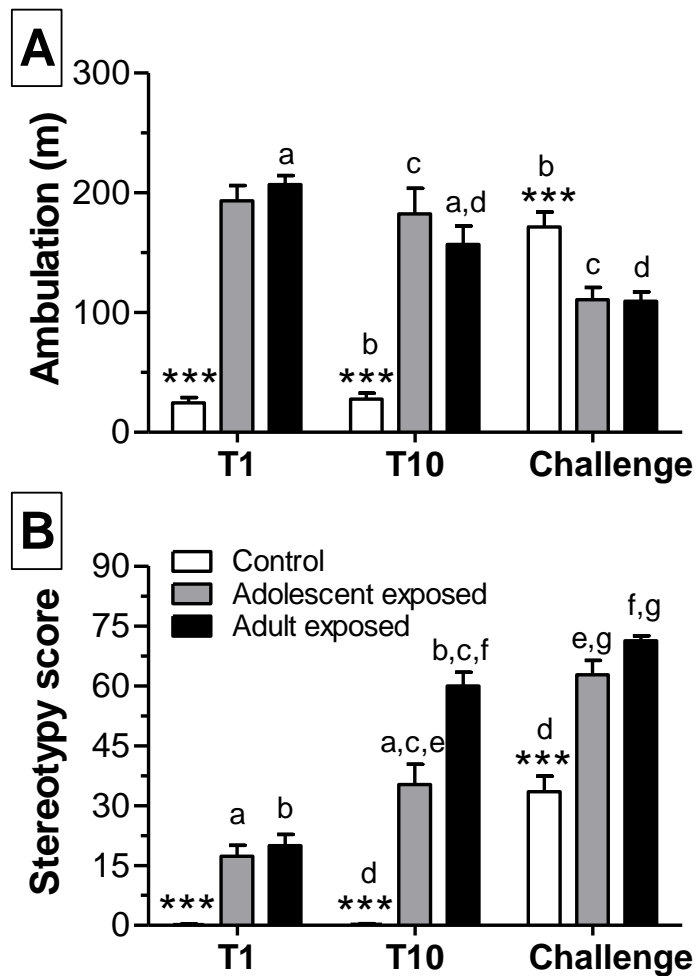
In conclusion, the results presented here suggest that repeated, intermittent exposure to amphetamine during adolescence has long-lasting consequences on drug sensitivity and cognitive function. While previous studies from our lab and others have demonstrated the negative consequences of psychostimulant exposure during adolescence (Counotte et al., 2009; Hankosky & Gulley, 2012; Harvey et al., 2009; Vorhees et al., 2005; Wiley & Burston, 2010), the present findings indicate that repeated exposure to amphetamine at this age produces long-lasting mnemonic dysfunction. Thus, it is likely that age-dependent differences in cognitive dysfunction following repeated exposure to amphetamine are the result of unique and persistent neuroadaptations in animals still undergoing neural development. Amphetamine-induced dysfunction in the PFC may be an important mediating factor in the observed cognitive impairments. This hypothesis will require further investigation, but it is noteworthy that adolescent development is marked by periods of altered receptor expression and signaling, increased synaptic pruning, and myelination in multiple brain regions, including the PFC (Andersen et al., 2000; Gould, Woolf, & Butcher, 1991;

Hamano et al., 1998; Kim & Juraska, 1997; Nunez, Nelson, Pych, Kim, & Juraska, 2000; Spear, 2000). Future studies employing neurophysiological and neuroanatomical methods are warranted to elucidate the specific neuroadaptations that accompany long-term cognitive dysfunction in animals exposed to amphetamine during adolescence.

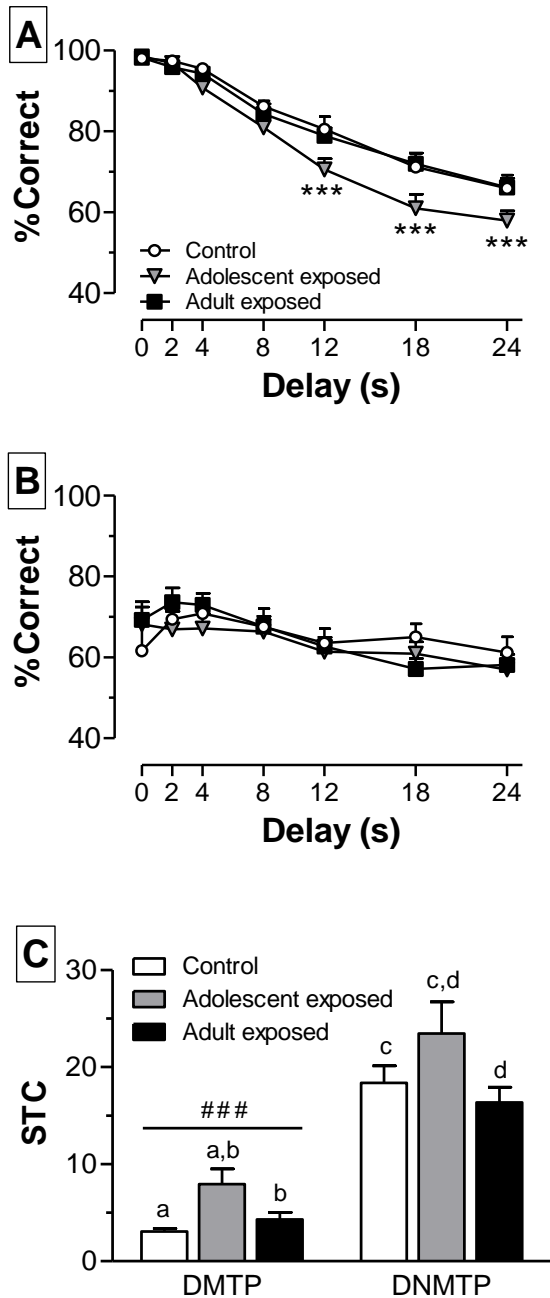
## Table and Figures

**Table 2.1.** Effects of amphetamine (AMPH) and ketamine challenges on choice latencies and the number of trials omitted during the working memory task in Experiment 1. Rats had a maximum of 10 s to respond on a lever during the choice phase before the trial was scored as an omission. The omission data also include trials wherein rats failed to respond in  $\leq 10$  s during the sample phase. Numbers in parentheses indicate the number of rats/group.  $^{\wedge}p < 0.001$ , compared to saline (omissions only, collapsed across exposure group);  $^{***}p < 0.01$ , compared to saline (latency and omissions, collapsed across exposure group).

Latency (s)	AMPH (mg/kg)				Ketamine (mg/kg)			
	Saline	0.3	0.75 <sup>^</sup>	1.25 <sup>^</sup>	Saline	5.0 <sup>***</sup>	7.5 <sup>***</sup>	10 <sup>***</sup>
Control (n=16)	0.67 $\pm$ 0.04	0.63 $\pm$ 0.05	0.57 $\pm$ 0.04	0.66 $\pm$ 0.04	0.62 $\pm$ 0.03	0.65 $\pm$ 0.04	0.61 $\pm$ 0.04	0.71 $\pm$ 0.04
Adolescent exposed (n = 15)	0.58 $\pm$ 0.04	0.55 $\pm$ 0.04	0.64 $\pm$ 0.05	0.56 $\pm$ 0.04	0.58 $\pm$ 0.04	0.69 $\pm$ 0.04	0.76 $\pm$ 0.04	0.67 $\pm$ 0.04
Adult exposed (n = 19)	0.46 $\pm$ 0.04	0.44 $\pm$ 0.04	0.51 $\pm$ 0.04	0.51 $\pm$ 0.04	0.46 $\pm$ 0.03	0.57 $\pm$ 0.04	0.61 $\pm$ 0.04	0.71 $\pm$ 0.04
Omissions (number/session)								
Control (n=16)	3.56 $\pm$ 5.91	11.4 $\pm$ 5.91	15.1 $\pm$ 5.91	41.8 $\pm$ 5.91	4.63 $\pm$ 9.06	21.3 $\pm$ 9.06	35.9 $\pm$ 9.06	58.7 $\pm$ 9.06
Adolescent exposed (n = 15)	0.27 $\pm$ 6.10	7.98 $\pm$ 6.52	30.8 $\pm$ 6.52	45.8 $\pm$ 6.52	0.53 $\pm$ 9.36	24.5 $\pm$ 10.0	39.2 $\pm$ 10.0	41.6 $\pm$ 10.0
Adult exposed (n = 19)	2.05 $\pm$ 5.42	0.90 $\pm$ 5.42	14.1 $\pm$ 5.42	40.3 $\pm$ 5.42	1.58 $\pm$ 8.31	38.0 $\pm$ 8.31	50.8 $\pm$ 8.31	60.5 $\pm$ 8.31

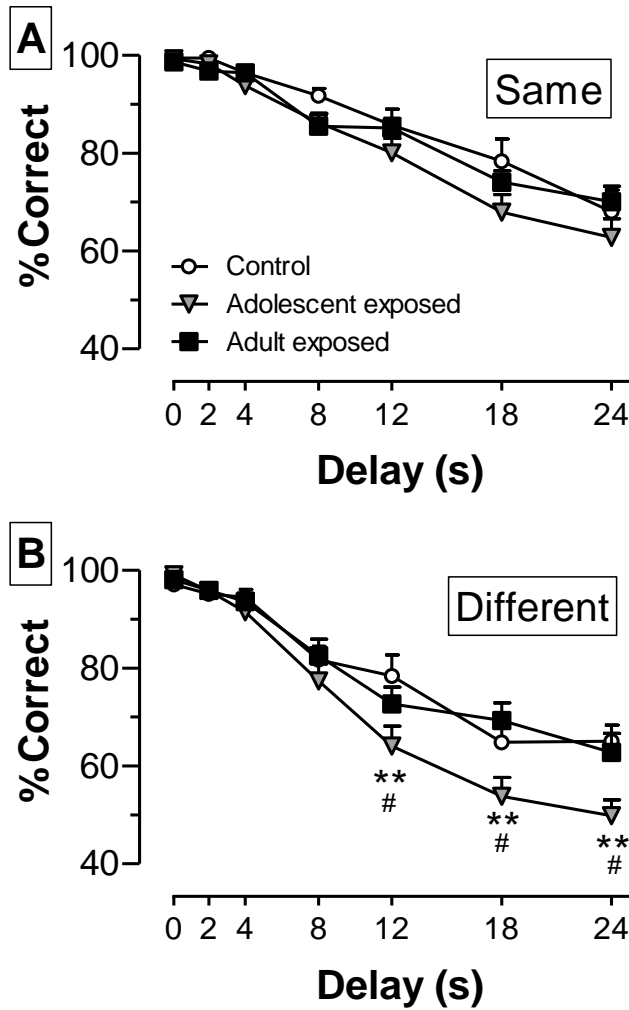


**Figure 2.1.** Ambulatory activity and stereotypy ( $n = 15-19$  rats/group) during the first (T1) and tenth (T10) saline or 3 mg/kg amphetamine (AMPH) treatment and after a challenge with 3 mg/kg AMPH, which was given after training and testing in the working memory task. For ambulation (A), data are presented as the mean cumulative activity during the 60 min after injection. For stereotypy, scores obtained every 5 min after injection were averaged to yield a single rating for the post-injection period. \*\*\* $p < 0.001$ , compared to AMPH-exposed groups within treatment day; matching letters indicate significant differences (ambulation:  $p < 0.01$ ; stereotypy:  $p < 0.01$ , for a,b,c,d,e and  $p < 0.05$ , for f,g).

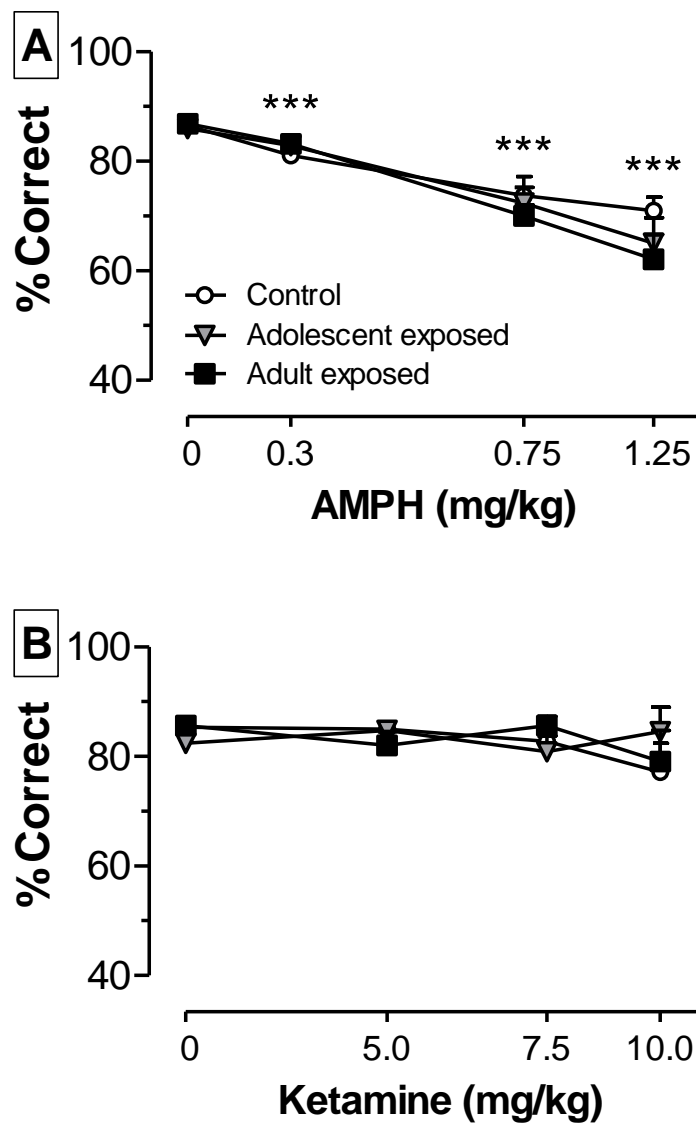


**Figure 2.2.** Performance on the working memory task in rats from Experiment 1 ( $n = 15-19/\text{group}$ ). Shown in (A) and (B) is mean choice accuracy (% correct) within each delay block averaged across the first two training sessions that any rat achieved the performance criterion. These were sessions 1-2 for DMTP and sessions 6-7 for DNMT. Shown in (C) is the mean number of sessions to reach a performance criterion (STC) of  $\geq 85\%$  correct choices for two consecutive sessions. Matching letters indicate  $p < 0.001$ ; \*\*\* $p < 0.001$  vs control and adult-exposed groups within delay; ### $p < 0.001$  vs DNMT, collapsed across exposure group.

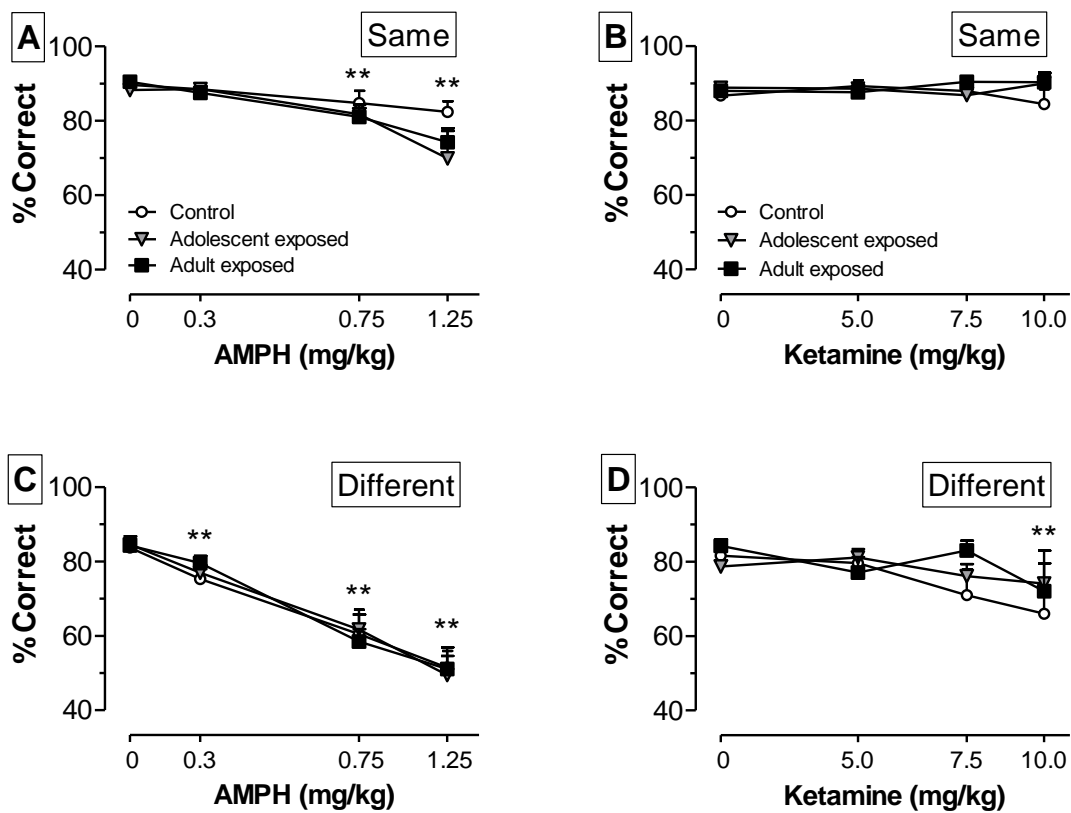




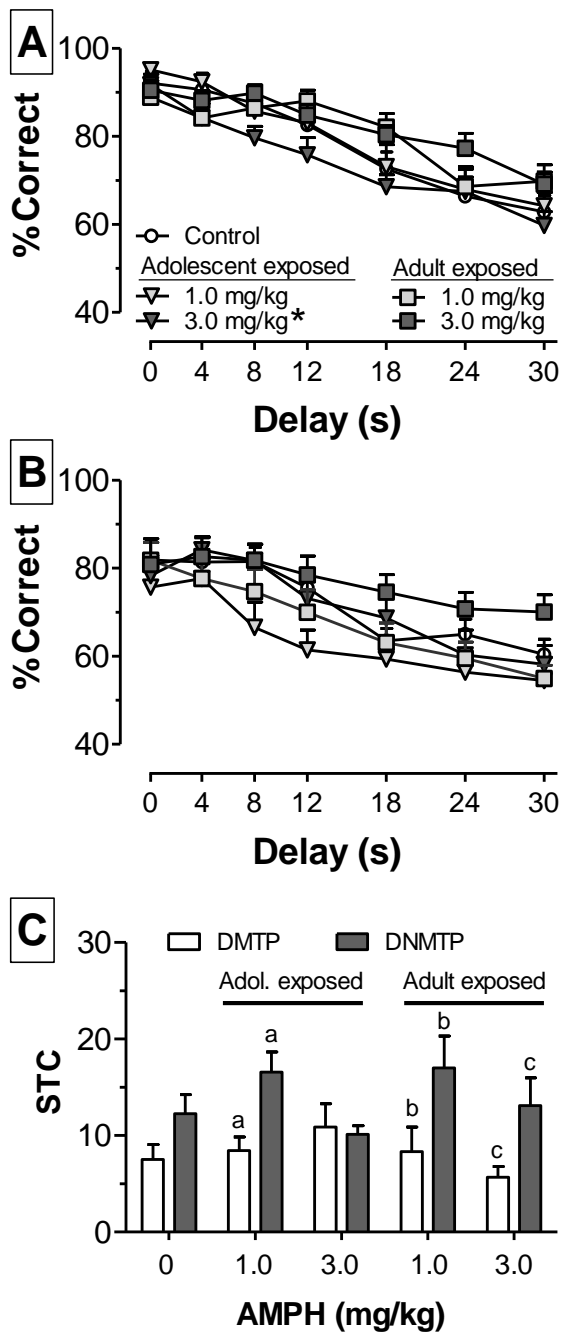
**Figure 2.3.** Proactive interference during DMTP training in rats from Experiment 1 ( $n = 15-19/\text{group}$ ). Delay-dependent performance was assessed across delay blocks in trials that required either the same correct response (A) or a different correct response (B) compared to the choice made on the immediately preceding trial. Shown are the group means within each delay block averaged across the first two training sessions on DMTP.  $**p < 0.01$  vs control;  $\#p < 0.05$  vs adult, within delay.



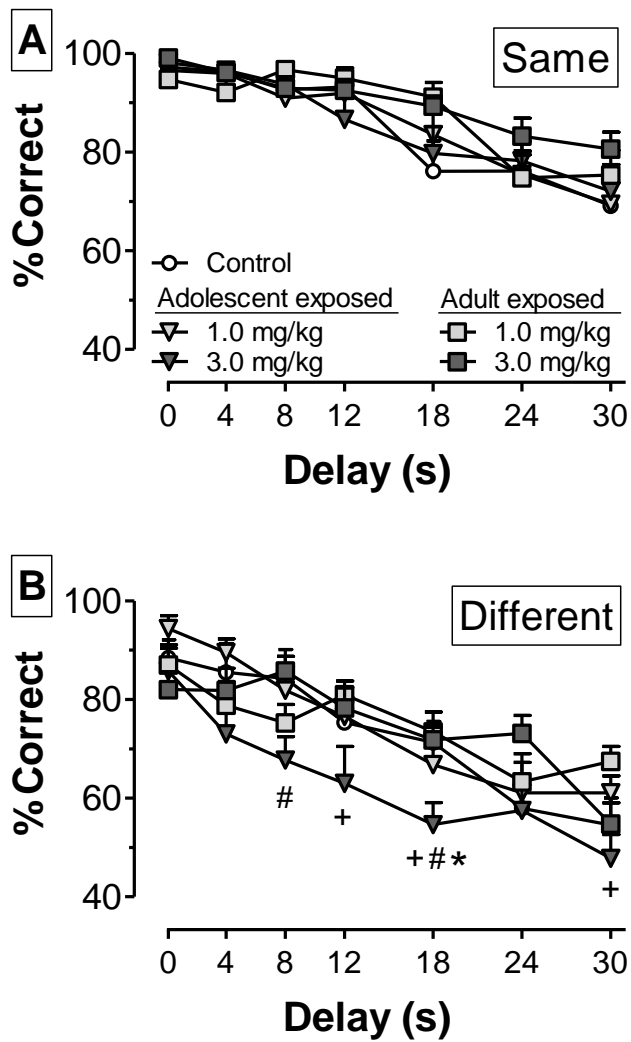
**Figure 2.4.** Effects of challenge injections with (A) amphetamine (AMPH) or (B) ketamine on task performance in Experiment 1 ( $n = 15-19$  rats/group). Drugs were administered i.p. 5-10 min prior to the start of DNMTF sessions. \*\*\* $p < 0.001$  vs 0 (saline), collapsed across exposure group.



**Figure 2.5.** Proactive interference during sessions following drug challenges in Experiment 1 ( $n = 15-19$  rats/group). Performance was assessed in trials that required either the same correct response or a different correct response compared to the choice made on the immediately preceding trial following (A and C) amphetamine (AMPH) and (B and D) ketamine. Shown are the group means for each dose tested.  $**p < 0.01$  vs 0 (saline), collapsed across exposure group.



**Figure 2.6.** Performance on the working memory task in rats from Experiment 2 ( $n = 8-10/\text{group}$ ). Data in (A) and (B) are presented as in Fig. 2.2. Because there were no significant effects of treatment on sessions to criterion (STC), data in (C) are plotted to emphasize differences in STC on DMTP compared to DNMT. Criterion performance was  $\geq 85\%$  correct for two consecutive sessions. Matched letters indicated significant difference ( $p < 0.05$ ); \* $p < 0.05$  vs adult-exposed (3.0 mg/kg) group.



**Figure 2.7.** Proactive interference during DMTP training in rats from Experiment 2 ( $n = 8-10/\text{group}$ ). Data were analyzed and presented as in Fig. 2.3  $^{\#}p < 0.05$  vs adult (3.0) within delay;  $^{+}p < 0.05$  vs adult (1.0) within delay;  $^{*}p < 0.05$  vs control within delay.

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### **Chapter 3. Amphetamine exposure during adolescence alters behavioral and prelimbic neuron responses to dopamine receptor agonist and antagonist drugs in adulthood.**

#### **Abstract**

Rationale: Repeated exposure to psychostimulants during adolescence produces long-lasting changes in behavior that may be mediated by disrupted development of the mesocorticolimbic dopamine system. Here, we tested this hypothesis by assessing the effects of amphetamine (AMPH) and dopamine receptor-selective drugs on behavior and medial prefrontal cortex (PFC) neuron activity. Methods: Adolescent rats were given saline or 3 mg/kg AMPH between postnatal day (P) 27 and P45. In Experiment 1, locomotor behavior was assessed during adulthood following challenges with a dopamine D<sub>1</sub> (SKF 82958) or D<sub>2</sub> (quinpirole) receptor-selective agonist. In Experiment 2, pre-exposed rats were challenged during adulthood with AMPH and a D<sub>1</sub> (SKF 83566) or D<sub>2</sub> (eticlopride) receptor-selective antagonist. In Experiment 3, the activity of putative pyramidal cells in the prelimbic cortex was recorded as rats behaved in an open-field arena before and after challenge injections with AMPH and one of the antagonists. Results: Compared to controls, adolescent pre-exposed rats were more sensitive to the stimulant effects of AMPH and the dopamine receptor agonists, as well as to the ability of the antagonists to reverse AMPH-induced stereotypy. Prelimbic neurons from pre-exposed rats were also more likely to respond to AMPH, primarily by reducing their activity, and the antagonists reversed these effects. Conclusions:



Exposure to AMPH during adolescence leads to long-lasting plasticity in the mesocorticolimbic dopamine system that likely mediates heightened response to the drug during adulthood and may also contribute to an increased incidence of cognitive dysfunction and substance use disorders in those who begin abusing drugs early in life.

## Introduction

Individuals with a long history of amphetamine (AMPH) misuse often exhibit impairments in tasks assessing executive cognitive functions such as impulse control, attention, working memory, and decision-making (McKetin and Mattick 1998; Ornstein et al. 2000; Woods et al. 2005; Monterosso et al. 2005; Casaletto et al. 2015). Notably, however, cognitive impairment is not an inevitable consequence of repeated AMPH exposure (Scott et al. 2007; Hart et al. 2011) and the development of drug-induced cognitive dysfunction may depend on a number of ancillary factors. One such potential factor is the initial age of drug exposure, with those beginning drug use during adolescence being most at risk. This hypothesis is supported indirectly by data showing that brain regions known to be important for cognition, including those in the corticolimbic circuitry such as the prefrontal cortex (PFC), amygdala, and hippocampus, are among the last to develop adult-like structure and function, and appear highly susceptible to environmental influences such as drug use (Paus et al. 2008; Gulley and Juraska 2013). In addition, cross-sectional analyses have suggested that those with the longest duration of psychostimulant abuse, which are individuals who started drug use in early adolescence, are the most susceptible to deficits in decision making (Rogers et al. 1999) and have a greater likelihood of developing a substance use disorder (Gilder et al. 2014; Wu and Schlenger 2003; Lopez-Quintero et al. 2011).

Studies using non-human animal models, which can experimentally manipulate age-of-exposure, suggest that adolescents are at a heightened risk of developing long-lasting cognitive dysfunction following chronic exposure to AMPH and other psychostimulants (Vorhees et al. 2005; Featherby et al. 2008; Harvey et al. 2009;

Counotte et al. 2011; Richetto et al. 2013; Hankosky et al. 2013; Hankosky and Gulley 2013; Hammerslag et al. 2014; Ye et al. 2014). For example, we recently demonstrated that rats given repeated, intermittent injections of 3 mg/kg AMPH showed delay-dependent deficits on an operant delayed matching-to-position task after a protracted drug free period (Sherrill et al. 2013). Notably, this working memory impairment was only found in animals that were given the drug during peri-adolescence. The same schedule of intermittent injections did not influence the performance of a separate group of animals that were exposed to AMPH in adulthood (Sherrill et al. 2013).

The mechanisms that underlie this enhanced vulnerability of adolescents to the adverse consequences of repeated AMPH exposure are uncertain, but a leading candidate is drug-induced changes in the normal development of the mesocorticolimbic dopamine system (Gulley and Juraska 2013). Studies in rodents have demonstrated that during adolescent development, there are significant changes in the density of monoamine transporters and dopaminergic fibers (Kalsbeek et al. 1988; Moll et al. 2000; Benes et al. 2000). Dopamine neurons in the ventral tegmental area (VTA), which project to multiple areas including the PFC and nucleus accumbens (NAc), are more active during the adolescent period compared to adulthood and this appears to be due to a relatively reduced GABAergic tone in the adolescent VTA (McCutcheon et al. 2012). In addition, there is an overproduction and subsequent decline of dopamine D<sub>1</sub> and D<sub>2</sub> receptor expression in the PFC and NAc as rats age from pre-adolescence into young adulthood (Andersen et al. 2000; Tarazi and Baldessarini 2000; Brenhouse et al. 2008). It has also been suggested that signaling via D<sub>1</sub>-D<sub>2</sub> heteromers, particularly in the striatum and NAc, is also unique in adolescents compared to adults and this may

contribute to enhanced vulnerability in adolescents to the effects of abused drugs (Perreault et al. 2014).

In the current study, we tested the hypothesis that repeated exposure to AMPH during adolescence leads to long-lasting changes in the function of D<sub>1</sub> and D<sub>2</sub> receptors in adulthood. In Experiment 1, we investigated if adult rats pre-exposed to AMPH in adolescence were sensitized to the motor-activating effects of the D<sub>1</sub>-selective agonist SKF 82958 or the D<sub>2</sub>-selective agonist quinpirole compared to saline-treated controls. In a second experiment, we tested if the D<sub>1</sub>-selective antagonist SKF 83566 or the D<sub>2</sub>-selective antagonist eticlopride would differentially influence AMPH-induced stereotypy in adolescent pre-exposed rats compared to controls. Lastly, in Experiment 3 we used *in vivo* electrophysiology in adolescent pre-exposed rats and controls to investigate functional changes in putative pyramidal cells of the prelimbic region of the medial PFC. The activity of these output cells is tightly regulated by D<sub>1</sub> and D<sub>2</sub> receptors (Seamans and Yang 2004) and we recently found that adolescent AMPH exposure alters D<sub>1</sub> receptor-mediated inhibition in these cells *in vitro* (Kang et al. 2016a; Paul et al. 2016).

## **Materials and Methods**

Subjects. The male subjects (n = 96 for all experiments) were offspring of male and female Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) bred in our animal facility. Following weaning at postnatal day (P) 22, rats were housed 2-3 per cage with food and water available *ad libitum*. Rats were maintained on a 12:12 hr light/dark cycle (lights on at 0800) and experimental procedures were conducted during the light phase. All experimental procedures were approved by the Institutional Animal Care and Use

Committee at the University of Illinois, Urbana-Champaign, and were in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011).

Open-field Apparatus. Locomotor activity was monitored in open-field arenas (41 x 41 x 41 cm) constructed of transparent acrylic walls and surrounded by photobeam frames (Coulbourn Instruments; Allentown, PA, USA) that recorded horizontal (lower frame; 2.5 cm above the arena floor) and vertical activity (upper frame; 15 cm above the arena floor). Each open-field arena was housed in a sound-attenuating cubicle (76 x 80 x 63 cm) that contained a 76 mm speaker fixed to one side wall to provide white noise (70 dB), two ceiling mounted white lights (4 W each), and a centrally mounted overhead camera (not used in this study). Computer software (TruScan v 2.01, Coulbourn Instruments) was used to record photobeam breaks and to calculate ambulation (m), rearing (number), and stereotypy. Stereotypy was measured as total number of repetitive movements, as defined by the recording software.

Drugs. D-amphetamine sulfate and SKF 82958 hydrobromide (D<sub>1</sub> agonist) were purchased from Sigma-Aldrich (St. Louis, MO, USA). SKF 83566 hydrobromide (D<sub>1</sub> antagonist), eticlopride hydrochloride (D<sub>2</sub> antagonist), and (-) quinpirole hydrochloride (D<sub>2</sub> agonist) were purchased from Tocris (Minneapolis, MN, USA). All drugs were dissolved in sterile saline (0.9% NaCl). Dosages were calculated based on the weight of the salt and given via intraperitoneal (i.p.) injection at a volume of 1 ml/kg.

Pre-treatment. At weaning, rats were assigned to exposure groups so that animals from 12 litters were represented similarly across groups. Cagemates (2-3 rats/cage) were assigned to the same treatment groups; controls were given 0.9%

saline (1 ml/kg) and rats in the pre-exposed group were administered AMPH (3 mg/kg). Beginning on P27, one injection was given every other day for a total of 10 injections. For each injection, rats were transferred from the colony to a testing room, and placed individually into acrylic tubs (46 x 25 x 22 cm) lined with hardwood bedding where they remained undisturbed for 60 min post-injection.

### *Experiment 1*

Approximately 30 days following their last pre-exposure injection (P70-P80), rats ( $n = 23$ ) were transferred from the colony to a separate testing room where they remained in their homecages for a 30 min acclimation period. Next, they were placed individually in an open-field arena for 15 min, then removed and injected with 1 ml/kg saline. They were immediately returned to the open-field and 30 min later challenged with either a dopamine  $D_1$  (SKF 82958, 1.0 mg/kg) or a  $D_2$  (quinpirole, 0.5 mg/kg) receptor agonist. Assignment of the specific agonist was made randomly. Rats were allowed to behave undisturbed for 90 min and then were returned to their homecage. Three days later, the drug challenge procedure was repeated, but rats received the dopamine receptor agonist they did not get during the first drug challenge. The order of drug challenges was counterbalanced within each group (i.e. control and pre-exposed).

### *Experiment 2*

Approximately 30 days following the last pre-exposure (P70-P80), rats ( $n = 51$ ) were given two challenge sessions that were separated by three days. During the first session, rats were placed individually in an open-field arena for 15 min, then removed and injected with 1 ml/kg saline (Injection 1). Thirty min later, they were administered 3

mg/kg AMPH (Injection 2) and allowed to freely move about the chamber for another 45 min. Lastly, rats were challenged (Injection 3) with saline, a dopamine D<sub>1</sub> antagonist (SKF 83566, 0.03 mg/kg), or a D<sub>2</sub> antagonist (eticlopride, 0.03mg/kg) and monitored for an additional 45 min. This procedure was repeated three days later during a second session. However, rats in the antagonist challenge groups were given the antagonist for Injection 3 that they did not receive during the first challenge session; the order of injections was assigned randomly. Rats previously challenged with saline on session one received saline again for Injection 3.

Data analysis for Experiments 1 and 2. In Experiment 1, ambulation, stereotypy, and rearing measures were summed in 15-min bins and analyzed using separate two-way ANOVAs, with time bin as the within-subjects factor and group (control, pre-exposed) as the between-subjects factor. Similarly, in Experiment 2, ambulation, stereotypy, and rearing measures were summed and analyzed using separate two-way ANOVAs with time bin as the within-subjects factor and between-subjects factors of group (control, pre-exposed). Cumulative stereotypy, which was obtained for each rat by summing this measure for the 45-min period following injections with AMPH and challenge drugs (i.e., saline or the dopamine receptor antagonists), was analyzed with three-way mixed factor ANOVAs with injection as the within-subjects factor and between-subjects factors of group (control, pre-exposed) and challenge drug (saline, SKF 83566, eticlopride). Main effects and interactions were further analyzed using Holm-Sidak multiple comparison procedures. Statistical analyses were conducted using R Statistical Computing Software (R: a Foundation for Statistical Computing; Vienna, Austria) or Systat 11 (Systat Software Inc.; San Jose, CA, USA).

### *Experiment 3*

Rats (n = 5/group) underwent surgical procedures for implantation of microwire electrode arrays between P65 and P70 using methods we described previously (Gulley and Stanis 2010). The array, which was implanted using stereotaxic coordinates for the prelimbic region of the medial PFC (3.0 mm AP, 0.7 mm ML, and 3.5mm DV to bregma; Paxinos and Watson 2007) consisted of eight Teflon-insulated stainless steel wires (50- $\mu$ m diameter each) arranged in a 2 x 4 pattern (NB Labs; Denison, TX, USA). Rats were allowed to recover from surgery for  $\geq 5$  days.

Between P70 and P80, which was approximately 30 days following the last pre-exposure injection, rats were transferred from the colony to a separate testing room, where they remained in their homecages for a 30 min acclimation period. During this time, voltage signals from each microwire were amplified with a unity gain field effect transistor (FET) headstage and transmitted via a shielded lightweight cable connected to a multi-channel commutator, which allowed the animal to freely move about the testing environment during recording sessions. Extracellular signals were amplified, band-pass filtered (250 Hz to 8 kHz), and digitally captured with a 40 kHz sampling rate using a Multichannel Acquisition Processor (Plexon Inc.; Dallas, TX, USA). Single-unit activity originating from putative pyramidal cells was identified based on waveform (i.e. spike) characteristics using well established criteria (Jung et al.; Barthó et al. 2004; Homayoun et al. 2005; Homayoun and Moghaddam 2006; Gulley and Stanis 2010). Spike sorting was also performed both online and offline from continuous data using both manual and automated procedures (Sort Client and Offline Sorter; Plexon Inc.).



Single-units were discriminated if the absolute refractory period of the target signal was > 1.1 ms and the waveform amplitude was at least 2.5:1 above background.

Rats were subsequently placed into an open-field arena for 15 min, then removed and injected i.p. with 1 ml/kg saline. Thirty minutes later, they were administered 3 mg/kg AMPH and allowed to freely move about the chamber for another 45 min. Lastly, they were injected with the D<sub>1</sub> antagonist SKF 83566 (0.03 mg/kg, i.p.) and monitored for an additional 45 min. During a second recording session, which took place two days after the first, the same procedure was used except the last injection given to rats was the D<sub>2</sub> antagonist eticlopride (0.03mg/kg, i.p.).

Experiment 3 data analysis. Electrophysiological data were imported into NeuroExplorer (NEX Technologies, Madison, AL, USA) and analyzed with custom scripts and methods similar to those used previously in our lab and others (Homayoun and Moghaddam 2006; Gulley and Stanis 2010). Neurons recorded during sessions 1 and 2 were treated as independent units, although the anchoring of the electrode connectors to the skull leaves the possibility that the same or similar population of cells were sampled across sessions. Baseline firing rates, which were designated as the mean activity (in Hz) during the saline phase of each recording session (15-min period after saline injection and before AMPH injection), were analyzed with two-way repeated measures ANOVA (group x session). The 99% confidence interval for this baseline period was then used to classify each unit's response to AMPH. A unit was categorized as "increased" or "decreased" following AMPH if its activity post-injection was above or below, respectively, the 99% confidence interval for (1) five of the nine 5-min recording bins, or (2) at least four consecutive 5-min bins post-injection (Gulley and Stanis 2010).

Those units not meeting one of these criterion were classified as “not changed”. The distribution of response types was compared between the control and pre-exposed groups using chi-square ( $\chi^2$ ).

To compare the magnitude change following AMPH challenge during sessions 1 and 2 combined, mean firing rate was calculated for each unit in 5-min bins and activity was normalized to each unit's baseline (mean firing during the 15-min saline injection period). This was done by dividing the unit's mean firing rate by its mean baseline firing rate and then expressing the value as a percentage. These data were subsequently analyzed with separate two-way repeated measures ANOVAs (group x time) for increased and decreased response types. The ability of D<sub>1</sub> and D<sub>2</sub> antagonists to alter AMPH-induced changes in firing rate were analyzed by comparing the mean normalized firing rate (% baseline) for the last 15 min of the AMPH injection interval to the last 15 min of the antagonist response interval. These data were then analyzed with separate two-way mixed factor repeated measures ANOVAs (group x injection) for each response type (increased or decreased) and antagonist (SKF 83566 or eticlopride). For all ANOVA tests, main effects and interactions were further analyzed using Holm-Sidak multiple comparison procedures where appropriate.

To analyze burst firing, spike train data were analyzed with the NeuroExplorer using the Poisson surprise method, which is robust against irregular patterns of activity and changes in mean firing rate that may obscure burst detection (Legédy and Salcman 1985; Homayoun et al. 2005; Homayoun and Moghaddam 2006). A minimum surprise value of 5 was set as the confidence level for detection. For each unit, bursting rate (bursts/min) and the percent of spikes in bursts were compared between groups

using separate two-way repeated measures ANOVAs (group x injection). Significant differences were followed-up with Holm post-hoc comparisons where appropriate. Statistical analyses for data obtained in Experiment 3 were conducted using SigmaPlot 12.5 (Systat Software Inc.; San Jose, CA, USA).

## Results

### *Experiment 1 – The effects of AMPH pre-exposure on D<sub>1</sub> and D<sub>2</sub> agonist challenge.*

Relative to baseline, there was an increase in motor activity following injection with the D<sub>1</sub> agonist SKF 82958 in both controls and AMPH pre-exposed rats (Fig. 3.1). For ambulation, we found a significant main effect of time bin ( $F_{8,168} = 16.7, p < 0.001$ ) and a significant group x time bin interaction ( $F_{8,168} = 6.30, p < 0.001$ ). As shown in Fig. 3.1a, rats pre-exposed to AMPH had a significantly greater maximal response that persisted for the entire 90-min post-drug interval. The D<sub>1</sub> agonist also increased stereotypy (Fig. 3.1b) and rearing (Fig. 3.1c) behavior. Separate ANOVAs indicated significant main effects of time bin for stereotypy ( $F_{8,168} = 5.79, p < 0.001$ ) and rearing ( $F_{8,168} = 4.54, p < 0.001$ ). However, in contrast to ambulation, there were no significant main effects of group or group x time bin interactions ( $ps > 0.05$ ).

During challenge sessions with the D<sub>2</sub> agonist quinpirole, rats pre-exposed to AMPH were more sensitive to drug-induced increases in ambulation (Fig. 3.2a). Two-way repeated measures ANOVA of these data revealed significant main effects of group ( $F_{1,21} = 6.76, p < 0.05$ ) and time bin ( $F_{8,168} = 97.5, p < 0.001$ ), as well as a significant group x time bin interaction ( $F_{8,168} = 4.97, p < 0.001$ ). Pre-exposed rats showed a significant increase in agonist-induced ambulatory activity beginning 30 min post-

injection, while the activity of control animals did not significantly change across the 90 min post-injection period. Similarly, analysis of stereotypy (Fig. 3.2b) indicated a significant main effect of time bin ( $F_{8,168} = 22.3$ ,  $p < 0.001$ ) and a significant group x time bin interaction ( $F_{8,168} = 6.88$ ,  $p < 0.001$ ). For rearing (Fig. 3.2c), there was a significant main effect of time bin ( $F_{8,168} = 10.1$ ,  $p < 0.001$ ) and a group x time bin interaction that was at the threshold for being considered statistically significant ( $F_{8,168} = 1.20$ ,  $p = 0.053$ ). Thus, for all three measures of motor activity, AMPH pre-exposed rats exhibited greater sensitivity to the effects of quinpirole.

*Experiment 2 – The effects of AMPH pre-exposure on AMPH and D<sub>1</sub> or D<sub>2</sub> antagonist challenge.*

In a separate group of rats, we assessed the effects of AMPH pre-exposure on the response to a challenge injection of AMPH and to subsequent injection with a D<sub>1</sub> or D<sub>2</sub> antagonist. As shown in Figure 3.3, a challenge injection of 3 mg/kg AMPH significantly increased motor activity regardless of pre-exposure; however, AMPH pre-exposed rats exhibited significantly less AMPH-induced ambulation than controls (Fig. 3.3a). A two-way repeated measures ANOVA on these data indicated significant main effects of group ( $F_{1,100} = 10.7$ ,  $p < 0.01$ ) and time bin ( $F_{5,500} = 179.9$ ,  $p < 0.001$ ), as well as a significant group x time bin interaction ( $F_{5,500} = 15.4$ ,  $p < 0.001$ ). This relative reduction in ambulation was likely a result of response competition, as AMPH-induced stereotypy was significantly greater in pre-exposed rats relative to controls (Fig. 3.3b). Two-way repeated measures ANOVA of stereotypy indicated significant main effects of group ( $F_{1,100} = 33.6$ ,  $p < 0.001$ ) and time bin ( $F_{5,50} = 60.3$ ,  $p < 0.001$ ), as well as a significant group x time bin interaction ( $F_{5,500} = 10.6$ ,  $p < 0.001$ ). Rearing behavior

followed a similar trend to ambulation, with pre-exposed rats rearing less than controls following AMPH (Fig. 3.3c). Two-way repeated measures ANOVA on these data revealed significant main effects of group ( $F_{1,100} = 14.9, p < 0.001$ ) and time bin ( $F_{5,500} = 88.9, p < 0.001$ ), and a significant group x time bin interaction ( $F_{5,500} = 20.4, p < 0.001$ ).

The effects of the dopamine receptor antagonists on AMPH-induced stereotypy were assessed following challenge injections with either saline, SKF 83566 ( $D_1$  antagonist), or eticlopride ( $D_2$  antagonist). As shown in Figure 3.4, the cumulative stereotypy response was greater in pre-exposed rats relative to controls, and continued to increase across the session in pre-exposed rats given saline at challenge. In rats given SKF 83566 (Fig. 3.4a) or eticlopride (Fig. 3.4b) at this challenge injection, however, this continued increase in stereotypy across the session was blocked. A three-way repeated measures ANOVA on these data indicated significant main effects of group ( $F_{1,96} = 8.09, p < 0.01$ ) and challenge ( $F_{8,768} = 136.0, p < 0.001$ ), and a significant group x challenge interaction ( $F_{16,768} = 1.71, p < 0.05$ ). There was no effect of challenge on stereotypy in controls that received saline during adolescence ( $p$ -values  $> 0.05$ ).

### *Experiment 3 – The effects of AMPH pre-exposure on medial PFC neuron activity.*

A total of 417 neurons were recorded from the medial PFC of control and AMPH pre-exposed rats ( $n = 5/\text{group}$ ) during two open-field challenge sessions. All electrodes were localized to the prelimbic region (Fig. 3.5a). During baseline (15 min prior to AMPH injection), we found no significant differences in mean firing rate across the two recording sessions so these data were collapsed. Moreover, the baseline activity of the

202 units recorded from controls ( $2.98 \pm 0.18$  spikes/sec) was not significantly different from that recorded in the 215 units from pre-exposed rats ( $3.17 \pm 0.17$  spikes/sec).

Cells were classified as increased, decreased, or not changed based on modulations in firing rate following AMPH challenge (Fig. 3.5b). In both control and AMPH pre-exposed rats, the majority of recorded prelimbic neurons (> 78%) were responsive to 3 mg/kg AMPH. Of these AMPH-responsive units, the most frequent response-type was a decrease in firing rate post-injection (Fig. 3.5c). We found a significant difference between groups in the population distribution of unit responses ( $\chi^2 = 13.56$ ,  $p < 0.01$ ). In pre-exposed animals relative to controls, there was a greater proportion of units that decreased firing rate after AMPH injection and a smaller proportion showing no change. The percentage of cells excited by AMPH was similar in the two groups.

Separate analyses of decreased and increased units revealed that the magnitude of change was greatest in the AMPH pre-exposed group (Fig. 3.6). For decreased units, there were main effects of group ( $F_{1,226} = 8.12$ ,  $p < 0.01$ ) and time ( $F_{11,2486} = 158.9$ ,  $p < 0.001$ ), as well as a group x time interaction ( $F_{11,2486} = 22.6$ ,  $p < 0.001$ ). For increased units, there was a main effect of time ( $F_{11,1353} = 18.0$ ,  $p < 0.001$ ) and a group x time interaction ( $F_{11,1353} = 2.12$ ,  $p < 0.05$ ). Between-group differences in response magnitude were significant by 20 min following AMPH injection and persisted for the duration of the recording period in units that had drug-induced decreases in firing rate (Fig. 3.6a). For those with drug-induced increases (Fig. 3.6b), where responses in pre-exposed rats were more variable, between-group differences were statistically significant by 25 min following injection.

At 45 min after they were administered AMPH, rats were given an injection of the D<sub>1</sub>-selective antagonist SKF 83566 (0.03 mg/kg) or the D<sub>2</sub>-selective antagonist eticlopride (0.03 mg/kg) to assess the ability of these drugs to alter AMPH-induced changes in medial PFC firing rate. As shown in Figure 3.7, data were analyzed by comparing the mean normalized firing rate during the last 15 min of the 45-min AMPH response interval to the same measure during the last 15 min of the 45-min antagonist response interval. For the D<sub>1</sub> antagonist, we found a significant main effect of injection ( $F_{1,99} = 10.9$ ,  $p = 0.001$ ) and a near-significant main effect of group ( $F_{1,99} = 3.43$ ,  $p = 0.067$ ) in decreased units (Fig. 3.7a); there was only a significant main effect of injection ( $F_{1,62} = 4.33$ ,  $p < 0.05$ ) for increased units (Fig. 3.7b). For the D<sub>2</sub> antagonist, we found a significant main effect of injection ( $F_{1,124} = 24.8$ ,  $p < 0.001$ ) and a near-significant main effect of group ( $F_{1,124} = 3.03$ ,  $p = 0.084$ ) in decreased units (Fig. 7a); none of the main effects or the interaction was significant for increased units (Fig. 3.7b). Thus, both antagonists tended to reverse AMPH-induced changes in firing rate, but this effect was most robust for the D<sub>1</sub>-selective compound SKF 83566 in units that decreased firing rate after AMPH. Moreover, the effect was similar in control and pre-exposed rats.

In addition to the analysis of firing rate, burst firing activity of prelimbic neurons was also assessed during each recording session. Separate two-way repeated measures ANOVAs for bursting rate revealed significant main effects of injection during both sessions (SKF 83566:  $F_{2,400} = 15.5$ ,  $p < 0.001$ ; eticlopride:  $F_{2,436} = 40.5$ ,  $p < 0.001$ ) and a significant group x injection interaction ( $F_{2,400} = 4.38$ ,  $p < 0.05$ ) for the first session when the D<sub>1</sub> antagonist was administered. As shown in Figure 3.8a, spontaneous bursting following saline injections in the first test session was blunted in pre-exposed

animals relative to controls. This effect of pre-exposure was evident in the second test session (Fig. 3.8b), but the group difference was reduced and not statistically significant. In both sessions, bursting rate tended to decrease following injections of 3 mg/kg AMPH, particularly in controls. AMPH-induced reductions in bursting rate were relatively unaffected by challenges with either antagonist.

Analysis of the percentage of spikes occurring in bursts also revealed effects of AMPH and antagonist challenges, but these varied depending on the recording session. In the session where the D<sub>1</sub> antagonist was tested, we found a significant group x injection interaction ( $F_{2,400} = 4.00, p < 0.05$ ). As shown in Figure 3.8c, prelimbic units recorded from pre-exposed rats had an increase in the percentage of spikes occurring in bursts following AMPH injection and this was reversed following injection with the SKF 83566. In the session where the D<sub>2</sub> antagonist was tested, we found a significant main effect of time ( $F_{2,436} = 14.0, p < 0.001$ ), and a near significant group x injection interaction ( $F_{2,436} = 2.97, p = 0.052$ ). Thus, in both groups there was a significant increase in the percentage of spikes occurring in bursts and this effect was reversed to a statistically significant extent in the pre-exposed group of rats.

## **Discussion**

The developing dopamine system of the adolescent brain may be an especially vulnerable target of the plasticity induced by repeated drug exposure. In the current study, we tested the hypothesis that AMPH exposure during adolescence induces changes in dopamine receptors that would persist into adulthood, lead pre-exposed rats to be more sensitive to the behavioral effects of AMPH and dopamine receptor-selective



drugs, and alter the function of putative pyramidal cells in the prelimbic region of the medial PFC. In Experiment 1, we found that AMPH pre-exposed rats, compared to controls, were more sensitive to the motor activating effects of the D<sub>1</sub> agonist SKF 82958 and the D<sub>2</sub> agonist quinpirole. With the D<sub>1</sub> agonist, this sensitization was isolated to ambulation, whereas the D<sub>2</sub> agonist induced a relatively greater effect on both ambulation and stereotypy. In Experiment 2, we found that pre-exposed rats were more sensitive to the stereotypy-inducing effects of AMPH as well as to the ability of a D<sub>1</sub> or D<sub>2</sub> antagonist to attenuate AMPH-induced stereotypy. Lastly, our electrophysiological recordings of prelimbic neurons (Experiment 3) revealed that cells recorded from pre-exposed rats, compared to those from controls, were less likely to be firing in bursts under baseline conditions. Following an AMPH challenge, these neurons were also more likely to be inhibited by AMPH, they exhibited a greater magnitude of change from baseline firing, and they were more sensitive to D<sub>1</sub> and D<sub>2</sub> antagonist-induced reversal of AMPH's effects on burst firing. Together, these results support the hypothesis that AMPH exposure during adolescence alters the development of the dopaminergic system, and in particular D<sub>1</sub> and D<sub>2</sub> receptor function, such that in adulthood animals are more sensitive to the neural and behavioral effects of drugs that influence dopamine receptor function.

It has been known for some time that rats pre-exposed to AMPHs in adulthood will exhibit behavioral sensitization to D<sub>2</sub>, but not D<sub>1</sub>, receptor-selective agonists following long withdrawal periods (Levy et al. 1988; Ujike et al. 1990; Vanderschuren et al. 1999). Here, we show that AMPH exposure during adolescence leads to a sensitized response to both D<sub>1</sub> and D<sub>2</sub> receptor-selective agonists when challenges are

given in adulthood. This suggests that drug exposure during adolescence, which is a developmental stage when these receptors are overproduced and subsequently pruned (Tarazi and Baldessarini 2000; Andersen et al. 2000; Brenhouse et al. 2008), may lead to unique changes in dopamine receptor expression and/or function compared to when drug exposure occurs during adulthood. Supporting this hypothesis, we recently showed that the same AMPH exposure protocol we used here induced a reduction of D<sub>1</sub> expression in the medial PFC (Kang et al. 2016b) and inhibited D<sub>1</sub> receptor-mediated inhibition of pyramidal cell output in the medial PFC (Kang et al. 2016a) when assessed in adulthood. Also consistent is our current finding that adult rats exposed to AMPH during adolescence were more sensitive to the effects of dopamine receptor-selective antagonists on AMPH-induced stereotypy.

Previous work revealed that when AMPH-exposed adolescents were given an AMPH challenge in adulthood, they exhibit sensitized locomotor responses (McPherson and Lawrence 2006; Mathews et al. 2011; Labonte et al. 2012; Richetto et al. 2013; Shanks et al. 2015) and stereotypy (Sherrill et al. 2013; Hankosky et al. 2013). However, it was not previously demonstrated that dopamine receptor antagonists could partially reverse this sensitized response in adolescent-exposed rats or that D<sub>1</sub> and D<sub>2</sub> receptor-selective antagonists were similarly efficacious. Activation of D<sub>1</sub>, and to a lesser extent D<sub>2</sub> receptors, is known to be important for the induction of AMPH sensitization (Ujike et al. 1989; Vezina 1996; Meng et al. 1998; Karper et al. 2002; Tanabe et al. 2004; Tournier et al. 2013; Kai et al. 2015), but their relative roles in its expression have been less well characterized. The fewer number of studies investigating expression have analyzed adult-exposed rats following relatively short

withdrawal periods ( $\leq 14$  days) and suggest a more prominent role for D<sub>1</sub> receptors (Shuto et al. 2006; Shi and McGinty 2011) or a D<sub>1</sub>-D<sub>2</sub> heteromer (Shen et al. 2015). The current findings are suggestive of an enhanced sensitivity of the developing adolescent brain to the ability of AMPH to induce enduring changes in the function of D<sub>1</sub> and D<sub>2</sub> receptors in the PFC, though this hypothesis will require further investigation with comparison groups of subjects that are outside the window of adolescent development when they are exposed to the drug (e.g., juveniles and adults).

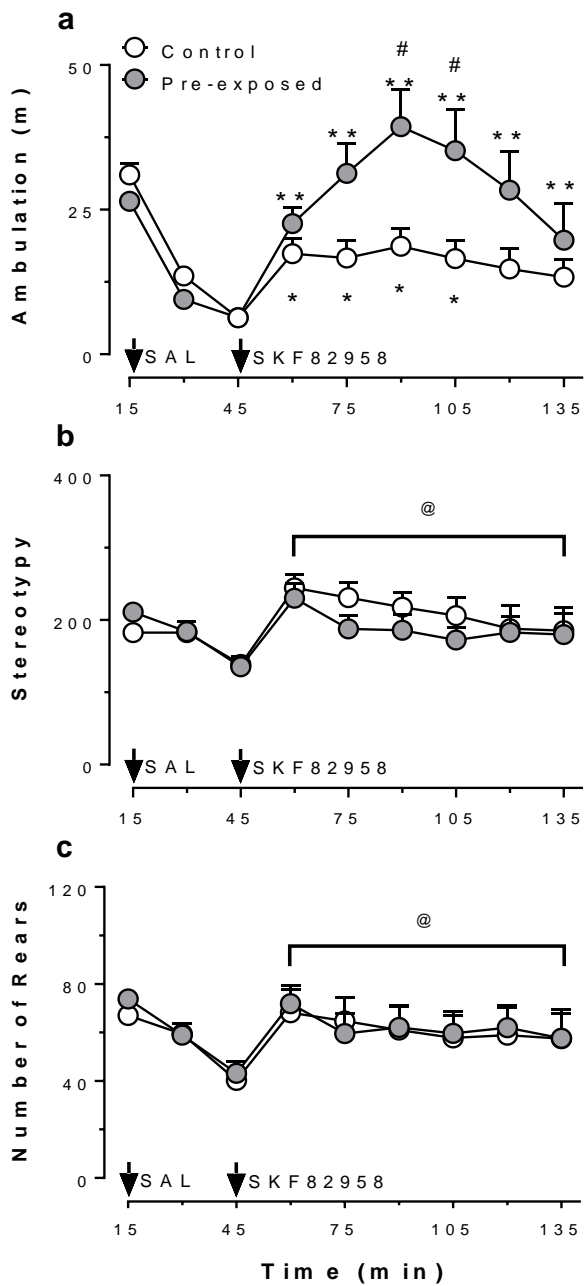
Our electrophysiology results suggest that AMPH-induced adaptations in the prelimbic cortex play a role in the behavioral adaptations we observed. Previously, neurons in the prelimbic and infralimbic regions of the medial PFC from AMPH-exposed adult rats were shown to be more responsive to AMPH compared to those recorded from controls when a challenge injection was given following a short withdrawal (Homayoun and Moghaddam 2006; Gulley and Stanis 2010). In these studies, which like the current experiments were done in freely behaving rats, the effects of AMPH pre-treatment differed depending on dose and withdrawal duration. In the earlier study (Homayoun and Moghaddam 2006), adult rats were treated for 5 days with 2 mg/kg AMPH or saline and were subsequently given a 2 mg/kg AMPH challenge following a 10-day withdrawal period. The authors reported that compared to controls, neurons from AMPH pre-exposed rats were more responsive to the challenge injection and the most frequent response observed was an inhibition in firing. In experiments from our laboratory (Gulley and Stanis 2010), adult rats were treated with 1 mg/kg AMPH for 5 days and challenged with the same dose following a 4-day withdrawal. Like the earlier study, AMPH pre-exposed rats were more responsive to AMPH and the predominant

effect was a decrease in firing rate and bursting. Here, where exposure to a higher dose of AMPH (3 mg/kg) occurred during peri-adolescent development and the AMPH challenge was given ~30 days later in adulthood, we observed a similar predominance of AMPH-induced decreases in firing rate. Additionally, we found that the prelimbic neurons from AMPH pre-exposed rats were more sensitive to D<sub>1</sub> and D<sub>2</sub> antagonist-induced reversal of AMPH's effects on burst firing, though the ability of these antagonists to reverse changes in firing rate were similar in pre-exposed and control rats. A similar reversal of AMPH's effects on neural activity in behaving rats was previously reported in recordings from dorsal striatum (Rosa-Kenig et al. 1993). Together, the previous and current findings suggest that the longer-term adaptations in medial PFC circuitry that AMPH induces likely involve changes in the sensitivity of D<sub>1</sub> and D<sub>2</sub> receptors and they may be more pronounced following adolescent exposure or a more protracted withdrawal period. Differentiating between these hypotheses of age or withdrawal duration dependency requires future studies, but our previous *in vitro* electrophysiology findings support the important role of exposure age for determining the effects of AMPH exposure on PFC neuronal physiology (Kang et al. 2016a; Paul et al. 2016).

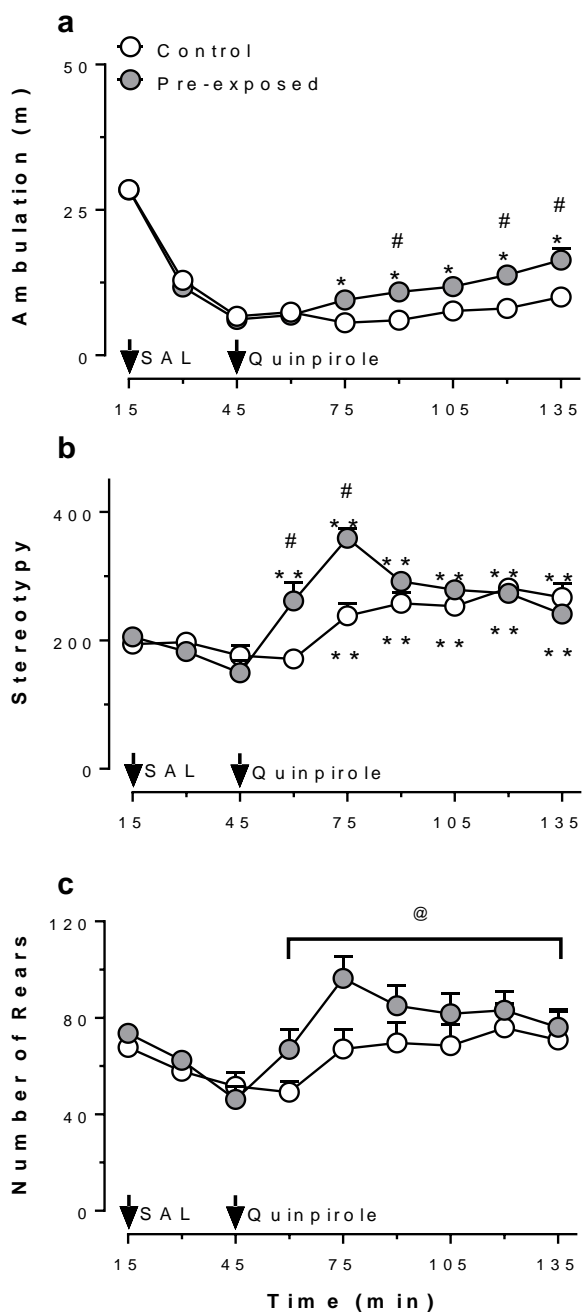
In summary, the results of the current study and others (Laviola et al. 2001; McPherson and Lawrence 2006; Labonte et al. 2012; Reynolds et al. 2015; Kang et al. 2016b; Tendilla-Beltrán et al. 2016) point to a key role for drug-induced changes in the structural and functional development of the mesocorticolimbic dopamine system in the neural and behavioral changes induced by adolescent AMPH exposure. One intriguing possibility, which awaits testing, is that the phasic dopamine changes caused by an

injection of AMPH have the unique ability to reorganize the structural and functional connections between the ventral tegmental area and the PFC in adolescents compared to adults. A recent optogenetic study revealed this potential mechanism for heightened mesofrontal circuit plasticity in adolescents compared to adults and further demonstrated that D<sub>2</sub> receptors play an important role in regulating this plasticity (Mastwal et al. 2014). Dopamine systems are undoubtedly not alone in being affected by AMPH, however, and long-lasting changes in GABAergic (Cass et al. 2013), glutamatergic (Counotte et al. 2011) and cholinergic (O'Dell 2009) functioning have also been reported following exposure to AMPHs and other psychostimulants during adolescence. An important goal of future studies will be to more specifically link drug-induced disruptions with the consequences they induce, especially those related to cognitive dysfunction and other behaviors known to be critically involved in addiction. It will also be important to provide more detailed analysis of the developmental timing of drug exposure as multiple recent studies have revealed that drug exposure during specific times during peri-adolescence can influence later neurophysiological and behavioral outcomes (Adriani et al. 2004; Kang et al. 2016b; Tendilla-Beltrán et al. 2016) and that puberty may influence drug effects on neuronal development differently in males and females (Drzewiecki et al. 2016; Juraska and Willing 2016).

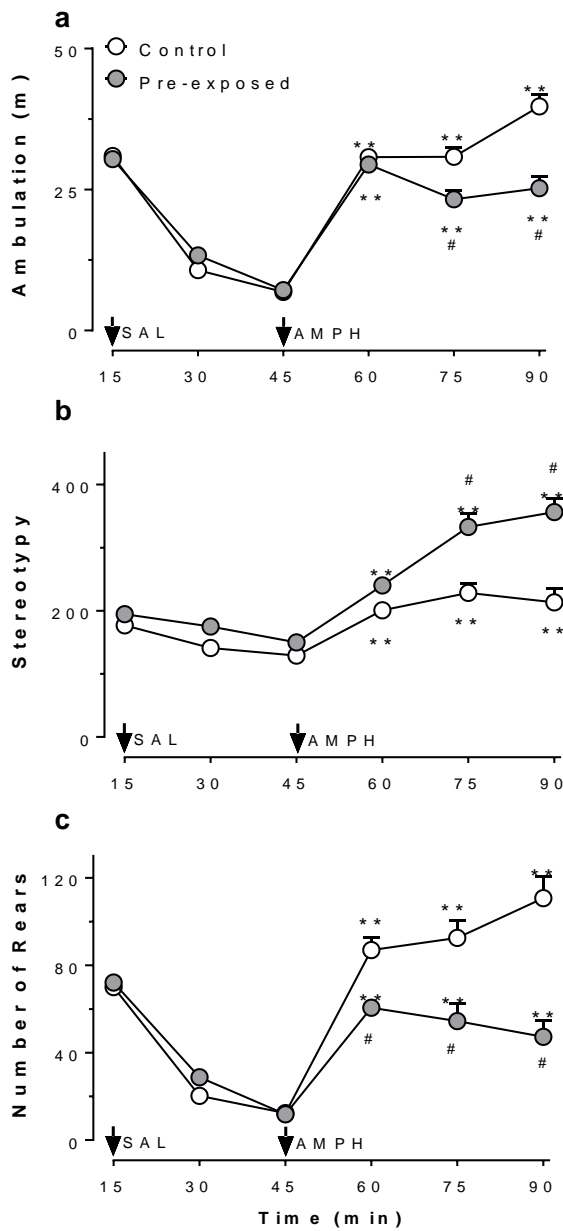
## Figures



**Figure 3.1** Ambulation (a), stereotypy (b) and rearing (c) in an open-field arena following challenge with 1.0 mg/kg SKF 82958 ( $n = 11-12/\text{group}$ ). Arrows indicate time-bins when rats were removed from the open-field and injected (i.p.) with saline and the  $D_1$  agonist. \* $p < 0.05$ , \*\* $p < 0.01$  vs baseline (45-min bin) within group; # $p < 0.05$  vs control within time bin; @ $p < 0.05$  vs baseline collapsed across group

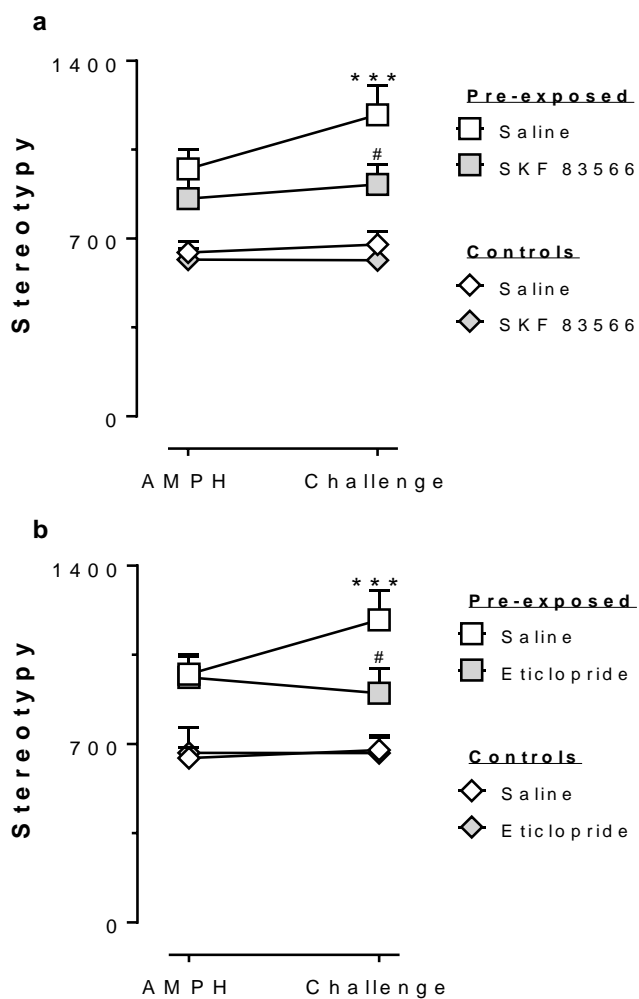


**Figure 3.2.** Ambulation (a), stereotypy (b) and rearing (c) in an open-field arena following challenge with 0.5 mg/kg quinpirole ( $n = 11-12/\text{group}$ ). Arrows indicate time-bins when rats were removed from the open-field and injected (i.p.) with saline and the  $D_2$  agonist. \* $p < 0.05$ , \*\* $p < 0.01$  vs baseline (45-min bin) within group; # $p < 0.05$  vs control within time bin; @ $p < 0.05$  vs baseline collapsed across group

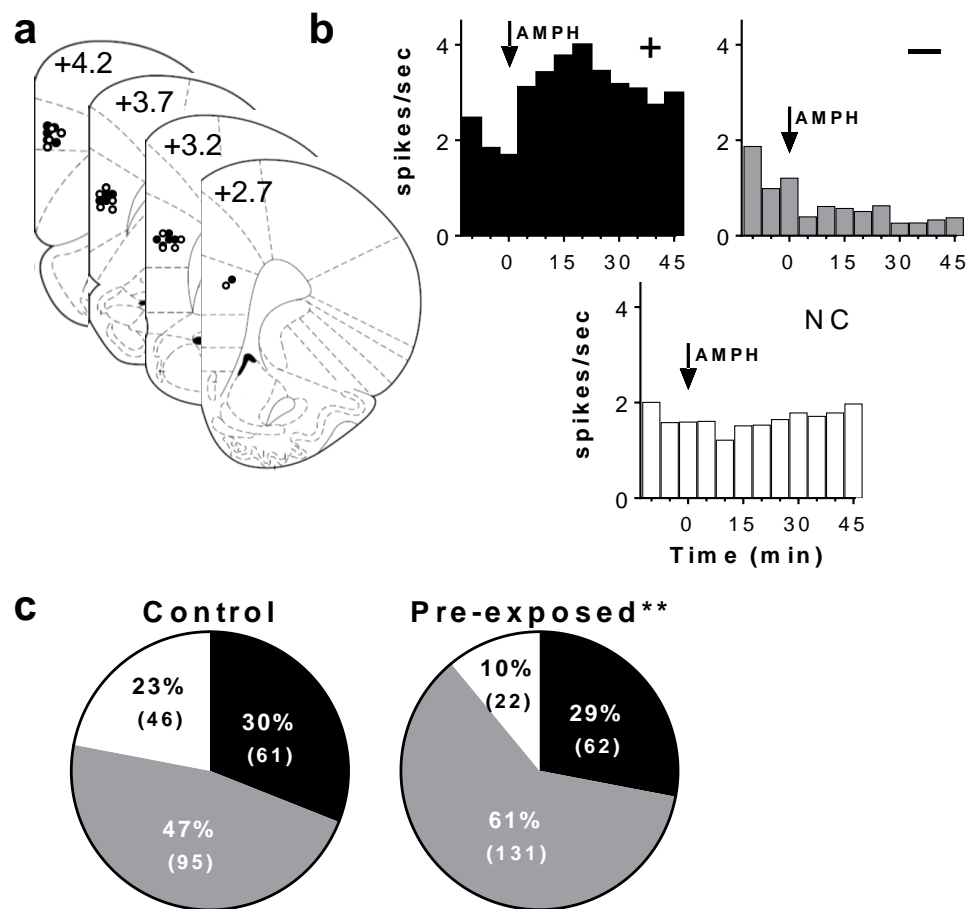


**Figure 3.3.** Ambulation (a), stereotypy (b) and rearing (c) in an open-field arena following challenge with 3.0 mg/kg AMPH. Arrows indicate time-bins when rats were removed from the open-field and injected (i.p.) with saline and AMPH. Data are presented collapsed across antagonist treatment group for pre-exposed rats ( $n = 25$ ) and controls ( $n = 26$ ). #  $p < 0.001$  vs control within time bin, \*\*  $p < 0.001$  vs baseline (45-min bin) within group

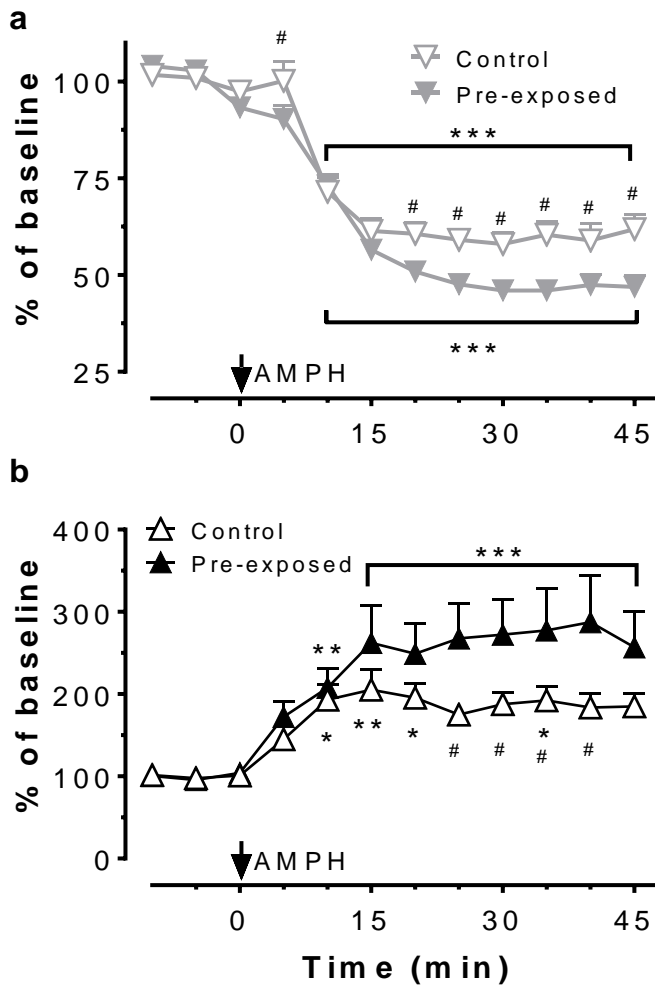




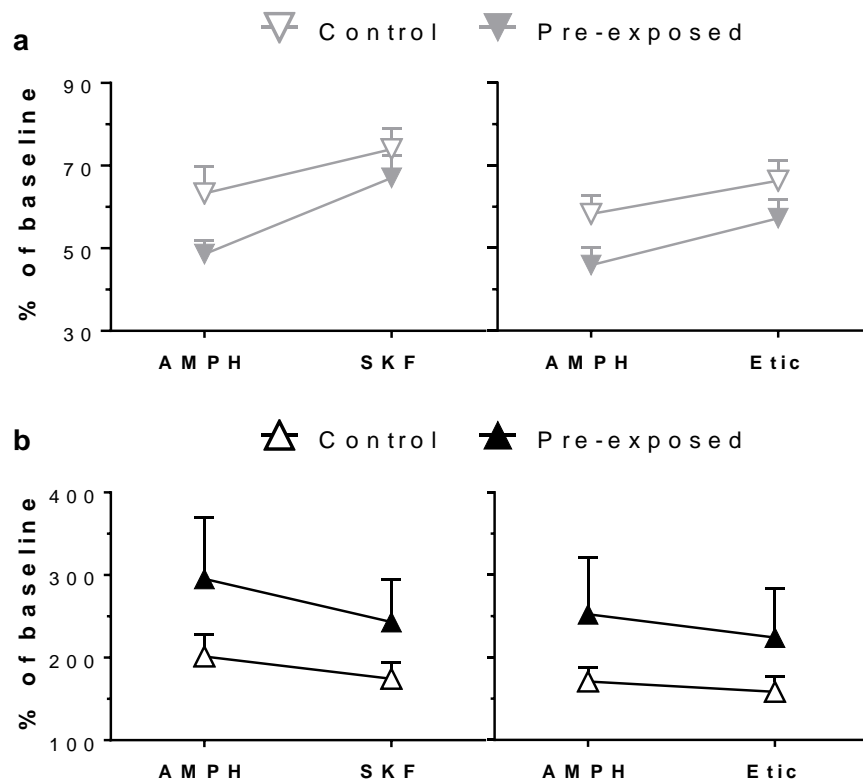
**Figure 3.4.** Stereotypy following 3.0 mg/kg AMPH and challenge injections of saline, 0.03 mg/kg SKF 83566 (**a**) or 0.03 mg/kg eticlopride (**b**). Data are presented as the cumulative response for the entire 45-min post-injection interval ( $n = 8-9/\text{group}$ ). \*\*\*  $p < 0.001$  vs AMPH period within group; #  $p < 0.05$  vs pre-exposed rats given a saline challenge



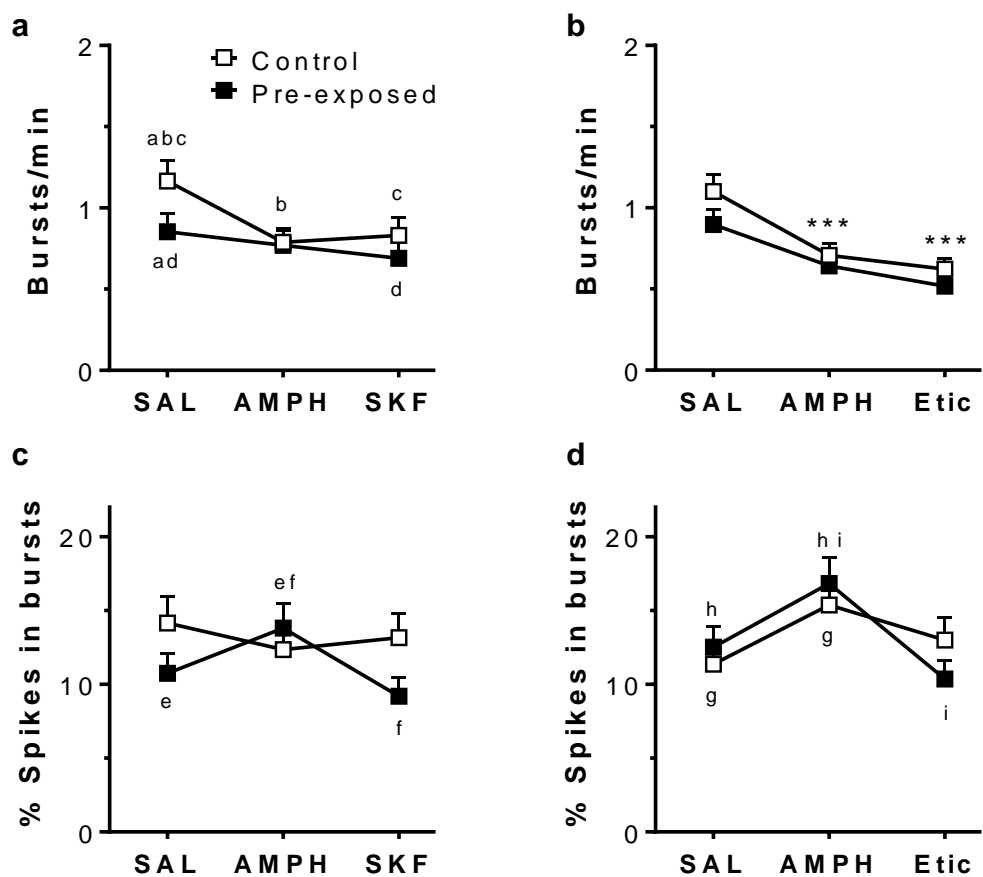
**Figure 3.5.** Putative pyramidal neurons from the prelimbic cortex that responded to a 3.0 mg/kg AMPH injection with an increase (+), decrease (-) or no change (NC) in firing rate. **(a)** A total of 417 recordings were made from electrodes implanted in the prelimbic region of the medial PFC. Closed circles represent approximate electrode tip locations for AMPH pre-exposed rats, whereas open circles represent locations for controls ( $n = 5$  rats/group). Numbers indicate the distance anterior to bregma (images adapted from Paxinos and Watson 2007). **(b)** Representative examples of each type of unit response (see Methods for classification procedure). Data are mean firing rate in 5-min bins during a 15-min saline injection baseline and 45 min after AMPH injection ( $t = 0$  min). **(c)** The population distribution of categorized units recorded from control and pre-exposed rats. The number of cells in each category is given in parentheses. Because there were no significant differences in the distribution of response types between recording sessions, data are presented collapsed across sessions. \*\* $p < 0.01$ ,  $\chi^2$  statistic comparing proportion of responses in control and pre-exposed groups



**Figure 3.6.** Magnitude of firing rate changes in prelimbic cortex cells classified as decreasing (**a**) or increasing (**b**) following a challenge injection of 3.0 mg/kg AMPH (designated by the arrow). Data are normalized to the pre-injection baseline for each unit. \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$ , vs. the last time bin before AMPH injection ( $t = 45$  min) within group; # $p < 0.05$  vs. pre-exposed group within time bin



**Figure 3.7.** Magnitude of firing rate changes in decreased (**a**) and increased (**b**) cells during the last 15-min of the 45-min recording period following 3.0 mg/kg AMPH injection and the last 15 min of the 45-min recording period following the D<sub>1</sub> antagonist SKF 83566 (0.03 mg/kg) or the D<sub>2</sub> antagonist eticlopride (0.03 mg/kg). Data are normalized to the pre-injection baseline for each unit.



**Figure 3.8.** Burst firing during saline baseline (SAL), following 3.0 mg/kg AMPH, and after challenge with the D<sub>1</sub> antagonist (SKF 83566; panels **a**, **c**) or the D<sub>2</sub> antagonist (eticlopride; panels **b**, **d**). Matching letters indicate significant between or within-group differences ( $p < 0.05$ ). \*\*\* $p < 0.001$  vs SAL, collapsed across group

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## **Chapter 4. General Discussion**

The objectives of this dissertation were threefold. The first was to identify if repeated exposure to AMPH has long-lasting effects on behavior supported by the medial PFC. The second was to determine if exposure to AMPH during adolescence produces outcomes distinct from those occurring when exposure occurs during adulthood. The last objective was to assess the impact of AMPH exposure during adolescence on dopamine receptor function and medial PFC neuron activity later in life. Findings described in Chapter 2 indicate that repeated exposure to AMPH has a long-lasting impact on psychomotor activity and working memory function. Interestingly, both adult- and adolescent-exposed animals displayed sensitization after extended drug-free periods, although, only rats given AMPH during adolescence showed impaired performance on the delayed matching to position task (DMTP). These results suggest that there is an age-dependent dissociation between the long-term effects of AMPH on adolescent and adult animals. The results described in Chapter 3 are consistent with the findings from Chapter 2, showing that animals exposed to AMPH during adolescence display long-lasting sensitization to AMPH. Additionally, those experiments indicate that adolescent-exposed animals have greater sensitivity to dopamine D<sub>1</sub> and D<sub>2</sub> receptor-selective drugs and display distinct differences in medial PFC neuron activity relative to drug-naïve animals. These neuroadaptations are likely to contribute to the behavioral outcomes associated with adolescent drug exposure and implicate the PFC and the interconnected mesocorticolimbic circuit as a target of enhanced plasticity during adolescence.

The PFC is part of an extended mesocorticolimbic network that plays a significant role in cognition and goal-directed behavior (Kolb 1984; Fuster 1993; Robbins and Arnsten 2009). On the bases of anatomical, neurochemical, and behavioral distinctions, the PFC is separable into medial and lateral subterritories, each with further subdivisions and numerous connections with other cortical and subcortical regions. In the rat, the medial PFC can be subdivided into a dorsal region, which includes the precentral (PrCm) and anterior cingulate (AC), and a ventral region comprised of the prelimbic (PL), infralimbic (IL) and medial orbital (MO) cortices (Leonard 1969; Krettek and Price 1977; Freedman and Cassell 1991; Granon et al. 2000; Heidbreder and Groenewegen 2003; Kesner and Churchwell 2011). Medial PFC subregions share reciprocal connections and also project to other cortical and subcortical areas involved in affective and sensory-motor processing. The major projections of the dorsal medial regions (AC/PrCm) are to dorsal striatum and various thalamic areas. The ventral medial PFC (PL/IL-MO) also shares connections with striatum and thalamus, but also sends efferents to the hypothalamus, amygdala, ventral tegmental area (VTA), and the core and shell of the nucleus accumbens (McGeorge and Faull 1989; Sesack et al. 1989; Hurley et al. 1991; Berendse et al. 1992; Bates and Goldman-Rakic 1993; Gorelova and Yang 1997; Seamans et al. 2001; Ding et al. 2001; Hoover and Vertes 2007).

The efferent projections of the medial PFC are primarily glutamatergic pyramidal cells, while its afferents are from more neurochemically diverse cells (Sesack et al. 1989; Hurley et al. 1991; Condé et al. 1995; Fisk and Wyss 1999). The medial PFC receives dense dopaminergic and GABAergic afferents from the VTA and projects back



to the midbrain with excitatory efferents (Lindvall et al. 1978; Beckstead et al. 1979; Pirot et al. 1992; Carr and Sesack 2000; Lewis and O'Donnell 2000). The medial PFC also receives glutamatergic inputs from the hippocampus and amygdala (Carr and Sesack 1996; Gabbott et al. 2002; Floresco and Grace 2003). These afferents form synapses on medial PFC pyramidal output neurons as well as local GABAergic interneurons (McDonald 1991; Condé et al. 1995; McDonald et al. 1996; Bacon et al. 1996; Gabbott et al. 2006). As such, afferent connections with the medial PFC may have an excitatory or inhibitory effect on local neuronal activity (Sesack et al. 1995; Mulder et al. 1997; Le Moine and Gaspar 1998; Floresco and Tse 2007).

While the striatum and VTA have received the most attention for their role in psychostimulant activity and sensitization, the PFC also plays a prominent role (Steketee 2003). The PFC exerts “top-down” control over subcortical neural circuits; signaling within and between limbic structures is influenced directly or indirectly by the PFC. For example, electrical stimulation of the medial PFC has the potential to directly excite or inhibit neurons within the ventral striatum via glutamatergic efferents (O'Donnell and Grace 1993, 1994) or indirectly via efferents to the hippocampus and VTA (Carr and Sesack 2000; Goto and O'Donnell 2002; Belujon and Grace 2008). Just as medium spiny neurons in the striatum display changes in excitatory activity following AMPH treatment (Haracz et al. 1989; Rosa-Kenig et al. 1993), so do pyramidal cells in the medial PFC (Gulley and Stanis 2010). Furthermore, cortical lesions attenuate AMPH-induced behavior and excitatory activity in the striatum (Tschanz et al. 1991; 1994). Dopamine transmission plays an integral role in signaling between limbic structures and the PFC (Pycock et al. 1980; Broersen et al. 1995; Cohen et al. 2002;

French and Totterdell 2002; Chudasama and Robbins 2004; Arnsten and Pliszka 2011). Top-down control exerted by the medial PFC is modulated by afferent dopamine projections (Granon et al. 2000; Hitchcott et al. 2007). Stimulation of dopamine neurons in the VTA increases extracellular concentrations of dopamine at terminals in the medial PFC and may inhibit local neural activity (Garris et al. 1993; Jay et al. 1995; Lewis and O'Donnell 2000). However, changes in mPFC activity depend on the location of synaptic terminals. Stimulation of dopamine D<sub>1</sub> receptors located on pyramidal cells tends to increase PFC neural activity (Henze et al. 2000; Lavin et al. 2005), whereas stimulation of D<sub>1</sub> receptors on local GABAergic interneurons produces inhibition (Seamans et al. 2001).

Dopamine signaling via D<sub>1</sub> and D<sub>2</sub> receptors is particularly important for both the acute and long-lasting effects of AMPH on psychomotor activity. Previous studies show that sensitization is blocked in adult animals administered dopamine D<sub>1</sub> or D<sub>2</sub> receptor antagonists prior to AMPH (Ujike et al. 1989; Vezina and Stewart 1989; Wolf and Xue 1999; Hall et al. 2009), and here we report attenuated expression of sensitization following adolescent AMPH exposure. AMPH produces age-dependent effects on glutamate and dopamine activity in the PFC (Kantak et al. 2007; Kindlundh-Hogberg et al. 2008; Mathews et al. 2011), and significant changes in neuron excitability and neurotransmitter release in animals exposed to the drug during adolescence (Laviola et al. 2001; McPherson and Lawrence 2006; Gramage et al. 2011). Excitatory activity of PFC pyramidal cells is tightly regulated by inhibitory GABAergic interneurons. During adolescence, inhibitory functioning in the PFC is reduced and dopamine receptors are in flux (Sturman and Moghaddam 2011; Brenhouse and Andersen 2011). Notably, D<sub>1</sub>

and D<sub>2</sub> receptors play a necessary role in this synaptic regulation. In an elegant set of recent experiments, repeated AMPH exposure during adolescence was found to reduce D<sub>1</sub> signaling and receptor expression in the medial PFC (Kang et al. 2016a; 2016b). These neuroadaptations were accompanied by attenuated inhibitory signaling later in adulthood. This may explain some of the changes in excitatory activity of pyramidal neurons recorded in the experiments described here. Interestingly, pharmacological manipulation of D<sub>1</sub> receptors following chronic exposure to AMPH has been shown to reverse cognitive dysfunction and neurophysiological adaptations in adult animals previously sensitized to the drug (Fletcher et al. 2007; Selemon et al. 2010). Nonetheless, D<sub>2</sub> receptors located on presynaptic interneurons also contribute to excitatory activity of pyramidal cells in the PFC. Given that rats pre-exposed to AMPH showed sensitivity to both D<sub>1</sub> and D<sub>2</sub> receptor selective drugs during young adulthood, neuroadaptations in D<sub>2</sub> signaling and receptor expression will be important to assess in future studies.

In recent decades awareness of the social problems and adverse health risks related to the pandemic abuse of AMPH has grown (Ujike et al. 1989; Sommers et al. 2006; Berman et al. 2009; Ho et al. 2009). Of particular concern, is accumulating evidence linking chronic AMPH abuse with psychological dysfunction (Scott et al. 2007; Grelotti et al. 2010; Dean et al. 2013). Epidemiological data indicate that adolescence is a time period associated with heightened vulnerability for the onset of psychiatric disorders including mood disorders, schizophrenia, and substance (Volkmar 1996; Grant and Dawson 1997; Pine 2002; Chambers et al. 2003; Casey et al. 2005). One of the core behavioral symptoms associated with these disorders is cognitive dysfunction,

while altered PFC function may be a unifying substrate. Previous studies show widespread structural and functional adaptations in the PFC following repeated AMPH treatment, with significant changes in dendritic complexity, neuronal excitability, and dopamine release (Heitz et al., 2003; Kindlundh-Hopberg et al., 2008; Gramage et al., 2011; Lee et al., 2011; Wong & Stevens, 2012). The findings presented here add to this collective, showing that exposure to AMPH during adolescence has a lasting impact on dopamine D<sub>1</sub> and D<sub>2</sub> receptor function and medial PFC neuron activity. It is not clear from these studies if the long-lasting effects of AMPH on the behavior of adolescent-exposed animals is solely a result of neuroadaptations in the PFC. It is unlikely that plasticity in only one node of the mesocorticolimbic circuit is responsible for the behavioral impact of AMPH. Indeed, evidence is accumulating that multiple areas in the mesocorticolimbic system undergo plasticity with AMPH exposure during adolescence. For example, the neural activity of VTA dopamine neurons remains altered in adult animals long after they were exposed to the drug during adolescence (Laviola et al. 2001; Labonte et al. 2012). Nonetheless, neuroadaptations in the PFC following repeated drug exposure during adolescence may be the primary substrate of cognitive dysfunction later in life.

Repeated exposure to AMPH has a robust and long-lasting effect on cognitive function (McKetin and Mattick 1997; 1998; Fletcher et al. 2005; Floresco and Whelan 2009). In both clinical and preclinical settings, the impairments associated with chronic AMPH exposure overlap with cognitive deficits found after damage or disruption of the PFC (Rogers et al. 1999). The medial PFC mediates attention and working memory functions necessary for appropriate decision-making and goal-directed behavior

(Mackintosh 1975; 1976; Honig and Thompson 1982). Previous studies indicate that chronic exposure to AMPH alters cellular structure and activity in the medial PFC (Robinson and Kolb 1997; Gulley and Stanis 2010). AMPH-induced plasticity in the medial PFC and corticolimbic circuitry is thought to mediate cognitive dysfunction associated with chronic exposure to the drug. Studies employing *in vivo* electrophysiology techniques have revealed that neurons in corticolimbic brain regions encode reinforcers, actions, and reward-paired stimuli (Nishijo et al. 1988; Apicella et al. 1991; Carelli and Deadwyler 1994; Schultz 2001; Hölscher et al. 2003; Roitman et al. 2005; Homayoun and Moghaddam 2006; Totah et al. 2009) and point to the medial PFC as a critical node within an extended network that functions to guide goal-directed behavior. Disruptions of medial PFC functioning lead to perseverative/habitual behavior (Killcross and Coutureau 2003; Marquis et al. 2007; Naneix et al. 2009; Furlong et al. 2010), and neurons within the medial PFC are activated during goal-directed behavior and serve to encode and update reward-related information (Homayoun and Moghaddam 2006; 2009). As shown here, AMPH given during adolescence impairs choice accuracy in the DMTP task. This effect is likely due to deficits in attention to sample stimuli and/or impairment in working memory function (Bushnell and Levin 1993; Stefani and Moghaddam 2002; Harper et al. 2005). Deficits on DMTP may be also related to issues of response inhibition, perseveration and cognitive flexibility. The relationship between working memory performance and flexibility has been described previously (Grégoire et al. 2012) and is noteworthy given recent reports from our lab showing that AMPH exposure during adolescence has a lasting and detrimental effect on cognitive flexibility and response inhibition (Hankosky et al. 2013; Hankosky and

Gulley 2013; Hammerslag et al. 2014). Nevertheless, while many reports indicate deficits in attention, decision-making, working memory, and reward-related learning, others find no change, or enhanced performance on cognitive tasks following drug exposure during adolescence (Kantak et al. 2014; Kirschmann et al. 2016; Spear 2016). Differences in drug dosing and other methodological discrepancies are likely to account for some of these conflicting findings (Grilly and Loveland 2001; Berman et al. 2009; Gulley and Juraska 2013). In addition, rearing history and the age at which animals are exposed to drugs are often not well controlled and many studies fail to describe the age of experimental animals during various stages of drug exposure, or carry out experiments with animals exposed to drugs and tested during adulthood.

The studies discussed in this dissertation demonstrate age-dependent effects of AMPH on behavior and suggest that adolescent exposure leads to long-lasting plasticity in the mesocorticolimbic system. One limitation of these studies is that the experiments were all performed with male subjects. Future studies are warranted to investigate whether female adolescent animals would have similar behavioral and neurophysiological outcomes following repeated exposure to AMPH. It's plausible that repeated AMPH exposure produces disparate outcomes in males and females given previous reports that highlight sex differences in neurodevelopment and drug-induced behavior across adolescence. In regards to the mesocorticolimbic system, the degree of maturational changes and the timing of those changes both vary depending on sex. For example, the number of neurons, synapses, and dendrites changes during adolescence, albeit, these changes are typically more robust and occur earlier in females relative to males (Markham et al. 2007; Koss et al. 2014; Willing and Juraska

2015; Drzewiecki et al. 2016). Thus, the timing of drug exposure during adolescence may lead to differential effects on females relative to males. Recent reports from our lab suggest there are no appreciable differences between males and females exposed to AMPH during adolescence in regards to executive function and affective behavior (Hammerslag et al, 2014; Kang et al, 2016b). Interestingly, exposure to AMPH before the onset of puberty had unique effects on dopamine D<sub>1</sub> receptor expression later in adulthood in both males and females despite the fact that females reached puberty approximately 5-10 days earlier than males (Kang et al, 2016b). Yet it is interesting that, as outlined in Chapter 2, exposure to AMPH possibly before and across puberty onset (see Kang et al, 2016b for mean age of pubertal onset of male SD rats) led to working memory dysfunction later in life. The relationship between pubertal timing and the effects of AMPH on cognition and PFC maturation are only beginning to emerge and it would be interesting to investigate this relationship in regards to the behavioral and electrophysiological outcomes reported in Chapter 3.

Sensitivity to AMPH-induced reward and locomotor sensitization appears earlier in females relative to males (Cirulli and Laviola 2000). In addition, females develop neurobiological and behavioral sensitization more rapidly than males (Camp and Robinson 1988). The precise mechanisms for these sex differences in AMPH-induced behavior are unclear, however, maturational differences in the mesocorticolimbic dopamine system between males and females have been documented previously. For example, changes in dopamine D<sub>1</sub> and D<sub>2</sub> expression are modest in females across adolescence, while both receptor subtypes undergo drastic overproduction and pruning in males (Andersen et al. 1997). Interestingly, manipulation of gonadal hormones at

puberty or earlier do not influence these sex-specific changes in dopamine receptor expression (Andersen et al. 2002). Nonetheless, sex differences in the development of the dopamine system may contribute to differences in AMPH-induced behavior between males and females. The results discussed in Chapter 3, demonstrate that in males, repeated exposure to AMPH leads to long-lasting locomotor sensitization to AMPH and dopamine-receptor selective drugs, as well as heightened sensitivity of PFC neurons to these drugs. The acute effects of adolescent AMPH exposure on monoamine transmission varies between males and females, and repeated exposure to AMPH leads to long-lasting sensitization of midbrain dopamine neurons in males (Labonte et al. 2012; Weiss et al. 2015). Sex differences in sensitivity to AMPH and neural maturation, warrant future studies to assess whether neurons in the mesocorticolimbic dopamine system of females are differentially sensitive to AMPH-induced plasticity compared to males. A further understanding of sex differences in drug-induced plasticity will be paramount to developing interventions for psychopathologies that arise in association with drug exposure during mesocorticolimbic maturation.



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