

The Effects of Methylphenidate Exposure During Distinct Developmental
Periods on the Rewarding Properties of Cocaine in Adulthood

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ABSTRACT

The Effects of Methylphenidate Exposure During Distinct Developmental Periods on the Rewarding Properties of Cocaine in Adulthood

Patrick N. Augustyniak

There has been controversy over whether early exposure to stimulant drugs, including methylphenidate (MPH), used in the treatment of attention deficit hyperactivity disorder (ADHD) increases the risk for drug abuse in adult life. This concern is based on research in adult animals showing that repeated exposure to stimulants results in sensitization to their rewarding effects. Yet, relatively little is known about the effects of developmental exposure to stimulants. Whether such effects vary between individuals with ADHD and others without the disorder is another question. In this thesis, I used place conditioning to examine whether MPH pretreatment alters the rewarding effects of cocaine later in life, first, in a typical strain of rats (Sprague Dawley) and, second, in an animal model of ADHD, the Spontaneously Hypertensive rat (SHR). Groups of male peripubertal rats were pretreated with MPH or saline for ten consecutive days. Twenty-five days later, the reward value of cocaine was assessed using place conditioning. Rats learned to associate cocaine with one of two dissimilar compartments. During the preconditioning phase, both MPH- and saline-pretreated rats spent equivalent amounts of time in each compartment. After conditioning, saline-pretreated rats given moderate and high doses of cocaine spent more time in cocaine-associated compartments than they did

before conditioning. However, in MPH-pretreated rats only the highest dose of cocaine established place preferences. These results suggest that peripubertal MPH administration decreases rather than increases the sensitivity to the rewarding effects of cocaine in both a typical strain of rats and an animal model of ADHD.

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The Effects of Methylphenidate Exposure During Distinct Developmental Periods on the Rewarding Properties of Cocaine in Adulthood

Of all the drugs that are abused by humans and self-administered by laboratory animals, psychostimulants such as cocaine are among the most potent reinforcers, and accordingly are much abused (Dackis & O'Brien, 2001; Giroud, Colassis, Rivier, & Ottinger, 1993; Withers, Pulvirenti, Koob, & Gillin, 1995). In addition to being one of the most abused drugs, it is now becoming clear that cocaine dependence may also be amongst the most serious and difficult addictions to treat due to its powerful addictive and rewarding properties (Hyman & Malenka, 2001; Leshner, 1997).

Oddly enough, certain psychostimulant drugs, for example, Methylphenidate (MPH: Ritalin®) are routinely prescribed to school children diagnosed with attention deficit hyperactivity disorder (ADHD), the most prevalent childhood disorder affecting 4-9% of youths (Wilens, Faraone, Biederman, & Gunawardene, 2003; Zuvekas, Vitiello, & Norquist, 2006). This despite the fact that little is known about how MPH acts to alleviate the symptoms of ADHD. The drug monograph specifies that: "There is neither specific evidence which clearly establishes the mechanism whereby Ritalin produces its mental and behavioral effects in children, nor conclusive evidence regarding how these effects relate to the condition of the central nervous system"(Novartis, 2006). What is more, MPH is now used by many preschoolers

as young as 3 years-old, even though MPH was not conceived nor tested for use in this cohort of children (Wilens, Faraone, Biederman, & Gunawardene, 2003). Indeed, nearly all psychotropic medications given to young children are prescribed in an “off-label” manner, which means that the drug was either meant for use in another population (i.e., not children) or intended for another ailment (Choonara, 2004). In a prospective study by Turner, Longworth, Nunn, & Choonara (1998) where the intended use of medications prescribed was assessed, they found that 36% of all medications prescribed to children are “off-label”. All of these facts have raised serious and valid concerns about the neurological effects of long-term psychostimulant use on the developing brain, notwithstanding the intended therapeutic benefits of such use. Particular attention has been focused on the effects that early exposure to psychostimulants may have on future use and abuse of drugs such as cocaine.

The goal of this thesis is to examine the consequences of early exposure to MPH with respect to the sensitivity to the rewarding properties of cocaine later in life. Before doing so, I will provide a brief background to key issues concerning addiction and the action of stimulant drugs.

Addiction and Sensitization

In the past, drug users and addicts were viewed and stigmatized as individuals lacking volition and judgment. Consequently, drug addiction was widely thought to reflect solely a behavioral problem (Leshner, 2001b; Wise, 2000). However, it has now become clearer that drug use in itself changes the

brain and disrupts adaptive decision-making. Based on this new knowledge, addiction has been recast as a brain disease (Leshner, 1997, 2001a; Wise, 2000) stemming, in part, from the extensive neuronal effects that addictive drugs have at the systems, structural, cellular, and genomic levels (Hyman & Malenka, 2001).

Drugs of abuse are habit-forming. That is, if one is exposed to a drug with abuse potential, the chances that one will re-approach the same drug are drastically augmented. This may happen because drugs produce pleasurable effects. The idea that drugs are used by virtue of their explicit hedonic properties was inherent in past addiction theories (Wise, 1982, T. E. Robinson & Berridge, 1993; Self & Nestler, 1995). However, such theories are now thought to be insufficient to explain addiction (T. E. Robinson & Berridge, 1993, 2000; Wise & Bozarth, 1987). For example, the hedonia, pleasure seeking concept cannot explain various behaviors and self-reports suggesting that after repeated drug use there is an apparent divergence between the hedonic value of the drug and the urge to consume it. That is, while the pleasurable effects (liking) diminish, drug craving and associated drug-seeking behaviors (wanting) persist and may even augment (S. Robinson, Sandstrom, Denenberg, & Palmiter, 2005; T. E. Robinson & Berridge, 1993, 2000, 2001). In addition, it has been shown that human addicts will self-administer sub-threshold drug doses over placebo despite having no conscious awareness of the hedonic effects of the drug (Fischman, 1989).

In an attempt to account for the many core features of addiction, novel theories have been proposed based on observations that while many drug effects

diminish (i.e., undergo tolerance) with repeated drug use, other crucial effects may actually augment. The common denominator of these theories is that particular drug effects and their neurochemical correlates undergo sensitization after repeated drug administration.

Much attention has been focused on the fact that following repeated intermittent exposure to a large class of drugs, notably psychostimulants including cocaine, amphetamine, and MPH, there is a significant increase in the behavioral activating effects of the drug (Kalivas & Duffy, 1993; Kalivas & Weber, 1988; Kuczenski & Segal, 1999; T. E. Robinson & Berridge, 1993). Experiments conducted to demonstrate behavioral sensitization commonly involve two phases. First, animals are pre-exposed to several injections of the drug or saline given daily or every other day. Second, following a brief drug-free period (5-10 days), all animals are given a challenge injection of the drug and locomotor activity is recorded. Interestingly, in response to the challenge injection, animals previously pretreated with the drug exhibit heightened behavioral activation compared to animals pretreated with saline. This demonstrates that the behavioral activating effects of drugs undergo sensitization as opposed to tolerance. The fact that behavioral sensitization may be an important feature of addiction is germane to the observation that most addictive drugs possess behavioral activating effects, and that these effects may indeed significantly influence the development of compulsive drug use (Wise & Bozarth, 1987).

Due to obvious methodological and ethical constraints on human research, the majority of studies on sensitization to abused drugs have been conducted in animals. The question of whether this phenomenon can also be observed in humans could, until recently, only be answered by anecdotal evidence suggesting that in humans, various drug effects, such as craving, seem to undergo sensitization after protracted drug use. A study by Strakowski, Sax, Setters, & Keck (1996) has shown that various outcome measures, such as number of eye-blinks and self-reports of "mania" undergo sensitization after just two intermittent sessions of exposure to oral d-amphetamine. Although the human literature is far from conclusive, there seem to be at least some evidence that behavioral sensitization does occur in human drug users.

Animal studies indicate that sensitization of the behavioral activating effects of psychostimulant drugs is accompanied by sensitization of their rewarding effects. That is, following repeated drug use the incentive motivational properties of the drug are enhanced as seen by an increase in drug-seeking behaviors (T. E. Robinson & Berridge, 1993, 2001, 2003). Evidence for such a phenomenon comes from self-administration and place conditioning studies.

The willingness of animals to lever press in order to procure an injection of a drug can be used to infer the incentive value of drugs. Typically, rats are trained on a simple reinforcement schedule such as a fixed ratio 1 (FR 1) in order to initiate stable responding. Once stable responding has been achieved, the effort required for an injection of drug can be gradually increased using a

progressive ratio schedule up to the point where animals cease responding. This point, known as the break point and defined as the maximal number of lever presses an animal is willing to emit in order to acquire a particular drug dose, can be used as a measure of the incentive motivational properties of drugs (Markou et al., 1993; Vezina, 2004; Vezina, Lorrain, Arnold, Austin, & Suto, 2002). Accordingly, many studies have shown that pretreatment with psychostimulants such as amphetamine, increase the amount of work (i.e., break point) an animal is willing to expend in order to get the drug. Thus, following pretreatment with the drug, its incentive properties undergo sensitization (Markou et al., 1993; Vezina, 2004). Mendrek, Blaha, & Phillips (1998) pretreated animals with either saline or amphetamine and then tested them for psychomotor sensitization. Following a 21-day drug free period, all animals were allowed to self-administer amphetamine on a progressive ratio schedule in order to determine between-group differences in break points. It was found that the drug-pretreated animals showed both enhanced behavioral responding (i.e., behavioral sensitization) and higher break points. It is noteworthy that the initial sensitized responses endured into the second phase of the experiment, conducted 21-days following pretreatment, lending further support to reports that sensitization can be very long lasting.

Place conditioning studies have also demonstrated that the incentive value of many addictive drugs undergoes sensitization. Place conditioning is a classical conditioning paradigm in which drug and saline are separately paired with distinctive environments. If, after pairing, the animal spends more time in the drug-paired environment, it can be said that the animal has developed a

place preference for that environment. The development of place preference is used as an index of the incentive properties of drugs. In a classic study by Lett (1989) it was shown that previous exposure to either cocaine, amphetamine, or morphine led to significant increases in the amount of time that the animals spend in the compartment associated with its respective drug. In addition to demonstrating sensitization of the incentive properties of various drugs through previous exposure to these drugs, Lett (1989) also showed that initial sensitization to one drug can carry-over and sensitize the incentive value of all of the other drugs she tested. That is, if an animal was pretreated with amphetamine, and then conditioning was conducted using morphine, sensitization to morphine was observed. This was true for any possible combination between the drugs tested. Cross-sensitization between various groups of drugs is now a well-established phenomenon (McDaid, Dallimore, Mackie, Mickiewicz, & Napier, 2005; T. E. Robinson & Berridge, 2003; Vezina, Giovino, Wise, & Stewart, 1989).

Place conditioning can also be used to gauge variations in the incentive properties of drugs by examining shifts in dose-response curves (DRC) after pre-exposure to various drug doses. One can ask the question: does previous exposure to a drug alter the dose that will be needed to produce place conditioning? Leftward DRC shifts following drug pre-exposure are indicative of a sensitized response since less drug is required to establish place conditioning. Shippenberg & Heidbreder (1995) demonstrated that previous exposure to cocaine produced leftward DRC shifts for place conditioning. In addition to shifting the DRC, previous exposure to cocaine also reduced the number of

required pairings between the environment and cocaine during conditioning in order to produce robust place conditioning.

Such findings, taken together with those from self-administration studies, firmly demonstrate that the incentive properties of many drugs undergo sensitization and that such effects in conjunction with psychomotor sensitization may indeed be important determinants in the transition from casual to compulsive drug-seeking and drug-taking behaviors observed in addiction.

Action of stimulant drugs & sensitization

Psychostimulant drugs exert profound neurobiological and behavioral effects when taken systemically. Specifically, drugs such as cocaine and amphetamine are readily and promptly absorbed into the brain via the bloodstream, where they act primarily at dopamine (DA), serotonin (5-HT), and norepinephrine (NE) transporters (Heikkila, Orlansky, & Cohen, 1975; Ross & Renyi, 1967; Ross & Renyl, 1967; Snyder & Coyle, 1969).

Although stimulant drugs administered systemically act on various brain regions and involve numerous neurotransmitters, they have been found to primarily exert their rewarding and behavioral activating properties by increasing synaptic DA levels within the mesocorticolimbic DA pathway (Carboni, Silvagni, Rolando, & Di Chiara, 2000; Di Chiara & Imperato, 1988; Vezina, 2004; Wise, 1998; Wise & Bozarth, 1987; Yokel, 1987). The mesocorticolimbic pathway is comprised of DA neurons projecting from the ventral tegmental area (VTA) to the NAcc, prefrontal cortex (PFC), and limbic

regions. DA produced in the VTA is released and binds to postsynaptic receptors in these terminal regions. The levels and duration of DA action in the synapse is controlled by a presynaptic active transport mechanism, the DA reuptake transporter (DAT), which is responsible for clearing DA out of the synaptic cleft, pumping it back into the cytoplasm (Gainetdinov & Caron, 2003).

Psychostimulant drugs such as cocaine increase DA levels in the mesocorticolimbic pathway by binding to the DAT and inhibiting reuptake, hence potentiating the actions of DA (Julien, 2005).

Evidence for the importance of DA in reward-related phenomena within the mesocorticolimbic DA pathway comes from various studies. For example, DA receptor antagonists such as pimozide significantly and dose-dependently reduce the reinforcing effects of cocaine (De Wit & Wise, 1977; Yokel & Wise, 1975). Similarly, neurotoxic lesions of the mesocorticolimbic DA pathway by 6-hydroxydopamine (6-OHDA) reduce the rewarding effects of cocaine and amphetamine (Roberts & Koob, 1982). It has also been found, using microdialysis, a procedure that enables the *in vivo* measurement of synaptic neurotransmitter levels, that stimulant drugs significantly increase extracellular DA levels in NAcc (Di Chiara & Imperato, 1988; Vezina, 1993).

In addition to the acute effects of psychostimulant drugs on DAergic functioning, which occur over minutes, these drugs have also been found to produce enduring alterations in neural functioning. Behavioral sensitization, for example, is accompanied by a sensitized midbrain DA response (i.e., enhanced drug-induced DA release) (Kalivas & Duffy, 1991; Kalivas & Stewart, 1991;

Wilcox, Robinson, & Becker, 1986; Wise & Bozarth, 1987). Repeated amphetamine and cocaine use also activate a vast number of proteins present in DA neurons and their targets that have been implicated in neural plasticity (Nestler, 1992, 2001, 2004; Self & Nestler, 1995). Indeed, following long-term stimulant exposure, morphological changes in neurons in the NAcc and PFC have also been documented by means of Golgi-staining (Li, Acerbo, & Robinson, 2004; T. E. Robinson & Kolb, 1997).

Evidence of long-lasting drug-induced neural plasticity matches the enduring behavioral consequences of drug use and may explain the persistence and high-rates of relapse that characterize addiction. Such evidence exemplifies the need and importance to further our understanding of the effects that drugs have on neural functioning.

Adolescence: a critical neurodevelopmental period

In recent years, it has become clear that there are behavioral and neurochemical differences in response to drugs as a function of age. Although there are many critical stages in the development of the brain, adolescence is of particular interest in the context of drug addiction since teenagers show an increased propensity to use and abuse drugs (Adriani et al., 2004; Adriani & Laviola, 2006; Adriani, Seta, Dessi-Fulgheri, Farabollini, & Laviola, 2003; Chambers, Taylor, & Potenza, 2003; Laviola et al., 2004; Tirelli, Laviola, & Adriani, 2003). For example, it has been documented that experimentation with and addiction to nicotine and many other illicit drugs occurs primarily during

adolescence (Giovino, 1999; Warner, Kessler, Hughes, Anthony, & Nelson, 1995). Additionally, it has been found that the initiation of drug use during this period is related to increased morbidity, psychiatric disorders later in life, and bleaker prognosis in cases where addiction persists into adulthood (Chambers, Taylor, & Potenza, 2003; Resnick et al., 1997).

Adolescence is a major transitional period between childhood and adulthood, during which hormonal (Romeo, 2003), neurochemical, and morphological changes in neurons are occurring at increased rates in order to transform the immature brain into a functionally and anatomically adult brain (Cameron, 2004; Dahl, 2004; Sisk & Foster, 2004). In an attempt to shed light on age-related differences on the effects of drugs, much attention has been given to the fact that adolescence, compared to adulthood, is characterized by impaired decision-making, impulsivity, risk-taking, and abnormal sensitivity to reward (Adriani & Laviola, 2004; Adriani, Seta, Dessi-Fulgheri, Farabollini, & Laviola, 2003; Chambers, Taylor, & Potenza, 2003). With the advent of advanced neuroimaging techniques, researchers have been trying to correlate deficiencies in judgment and decision-making with alterations in brain activity (e.g., see Bjork et al., 2004; Volkow & Li, 2005).

In an attempt to consolidate the plethora of findings in this area, Ernst, Pine, and Hardin (2006) have proposed the Triadic model which states that the risky behaviors of adolescents can be explained by a neurobiological imbalance between the approach/reward and avoidance/inhibitory ("brake") neural systems during this period of development. The model is based on converging

evidence suggesting that, in general, the ventral striatum and amygdala are involved in opposite functions, reward-driven approach and harm avoidance, respectively (Chambers, Taylor, & Potenza, 2003; Tzschentke, 1998; Weiss, 2005; Weiss et al., 2001). During normal functioning of the mature brain, various frontal cortical regions such as the medial and ventral prefrontal cortex (PFC) regulate and balance the activity of the reward and avoidance systems. The suggestion is that adolescents often exhibit aberrations in reward and risk/avoidance motivated behaviors because of an underdeveloped PFC that, in turn, results in over-activation of the reward system and under-activation of the avoidance system.

Even though a clearer understanding of the mechanisms involved in the increased propensity to addiction during adolescence seems to be emerging, results must be viewed with caution since there remain many questions to answer and important disparities exist in current explanations of "risky" behavior. For example, while researchers such as Ernst et al. (2006) argue that during adolescence the reward related brain mechanisms are overactive, others argue exactly the opposite. Drawing on a vast collection of both human and animal research, Spear (2000) concluded that adolescents actually have an under-active/underdeveloped reward system. Briefly, it is argued that since adolescents have an under-active reward system they exhibit problems with delayed reward and require powerful rewards in order to stimulate the system to levels comparable to those of adults. It follows then, that risky behavior may ensue from the incessant need to stimulate an under-active reward system.

Although risk-taking and impulsive behaviors during adolescence can undoubtedly be associated with heightened risks for drug-use, there may also be potential differences in how drug-relevant neural circuitry responds to drugs during this period. In fact, it is known that adolescents exhibit differential neural sensitivity in response to various drugs (Laviola, Wood, Kuhn, Francis, & Spear, 1995; Tirelli, Laviola, & Adriani, 2003). For example, differential effects of peri-adolescent versus adulthood drug pretreatment were recently demonstrated in a study by Adriani et al. (2003) in which adolescent and post-adolescent rats were pretreated with either nicotine or saline and then the rewarding effects of nicotine were assessed later in adulthood. The results showed that adolescent versus post-adolescent nicotine pretreatment significantly enhanced the rewarding effects of nicotine later in the adult as measured by the total volume of self-administered intravenous nicotine. Results such as these lend credence to the observation that adolescence is a period of enhanced vulnerability to the addictive properties of drugs and that sensitization to the rewarding effects of drugs during this period may increase the chances of pathological drug use later in life.

Going back to the issue of pediatric psychostimulant use, the previous discussion leaves open such vexing questions as to whether long-term stimulant use predisposes youths to later addiction. The use of animal models could contribute significantly to the understanding of how long term stimulants affect the developing brain. As a first step toward this endeavor, the following experiments investigate the effects of peripubertal MPH exposure on future responsiveness to cocaine.

Experiment 1

With the dramatic upsurge of MPH prescriptions for the treatment of ADHD in the past decade, much attention has been given to this increasingly controversial practice. MPH is the preferred and most common form of treatment for ADHD. Although there is no question about its effectiveness, concerns have been raised due to the fact that the drug's pharmacological actions closely parallel those of cocaine (Greenhill, Halperin, & Abikoff, 1999; Greenhill et al., 2002). For example, MPH, like cocaine, increases synaptic DA levels by binding to DAT and blocking DA reuptake, an effect that has been demonstrated in various species including humans (Gatley et al., 1999; Julien, 2005; Volkow et al., 1999; Volkow, Fowler, Wang, Ding, & Gatley, 2002). However, until recently, little was known about the behavioral and neural consequences of peripubertal MPH exposure, especially with regard to the propensity of developing drug addiction in later life.

Although the rate at which MPH gets into the brain may be an important factor in mediating its rewarding effects, Volkow et al. (1998) found that MPH taken orally by healthy human subjects lead to a greater than 50% DAT occupancy, demonstrating that MPH much like cocaine, is extremely effective at blocking DAT. In a follow-up imaging study Volkow et al. (2001) showed in both humans and baboons that therapeutic doses of oral MPH increased striatal DA levels significantly.

It has been well established that, in adult rodents, repeated exposure to psychostimulants induces sensitization which is paralleled by molecular, cellular, and morphological neuroadaptations, some of which are thought to underlie the compulsive nature of addiction (Kalivas & Duffy, 1990, 1993; Kalivas & Stewart, 1991; T. E. Robinson & Berridge, 2001, 2003; T. E. Robinson & Kolb, 1997). Repeated exposure to MPH has also been shown to produce sensitization as measured by increased locomotor activation and leftward DRC shifts in place conditioning studies (Crawford, McDougall, Meier, Collins, & Watson, 1998; Gaytan, al-Rahim, Swann, & Dafny, 1997; Meririnne, Kankaanpaa, & Seppala, 2001; Sproson, Chantrey, Hollis, Marsden, & Fonel, 2001). In addition, once sensitization to MPH has occurred, cross-sensitization to other stimulant drugs, including amphetamine and cocaine, has been documented. For example, a study by Schenk & Izenwasser (2002) demonstrated that pretreatment with high doses of MPH significantly increased the rate at which animals learned to self-administer cocaine. This finding suggests that MPH exposure produces sensitization to the rewarding effects of cocaine.

Surprisingly, although there is ample evidence about the effects of MPH treatment during adulthood, little is known about the long-term consequences of exposure to MPH during development. In a recent study by Yang, Swann, & Dafny (2003) it was shown that regardless of the age at which MPH pretreatment occurred (PND 35 versus PND 60), repeated MPH exposure sensitized the locomotor activating effects of amphetamine. Similarly, Brandon, Marinelli, Baker, & White (2001) showed that adolescent rats (post natal day; PND 35) that were pretreated with a low (i.e. therapeutically relevant) dose of MPH for seven

days showed a sensitized response to the rewarding effects of cocaine as measured by the self-administration paradigm during adulthood (PND 56). Interestingly, a longitudinal follow-up study on children diagnosed with ADHD found that in adulthood they were at an increased risk for abusing various drugs including cocaine, amphetamine, and nicotine, and that abuse of these drugs is related to stimulant treatment during childhood (Lambert & Hartsough, 1998). The results of these studies are in accordance with the sensitization view, and are indicative of an increased propensity to addiction in adulthood by virtue of the fact that MPH treatment appears to sensitize the rewarding effects of abused drugs.

In contrast to the above mentioned studies suggesting an increased propensity for drug abuse after early MPH treatment, other studies have actually found that that MPH treatment in ADHD significantly reduces the risk for substance abuse in adulthood. In particular, these studies show that although children diagnosed with ADHD are at a greater risk for drug abuse in adulthood, taking MPH reduces this risk (e.g., Biederman, 2003; Biederman, Wilens, Mick, Spencer, & Faraone, 1999; Mannuzza, Klein, & Moulton, 2003; Spencer, Biederman, & Wilens, 2004; Wilens, Faraone, Biederman, & Gunawardene, 2003). Andersen, Arvanitogiannis, Pliakas, LeBlanc, & Carlezon (2002) used a rodent model, to explore the possibility that early exposure to MPH makes drugs of abuse less rewarding later in life. The rewarding effects of cocaine were assessed using place conditioning. They demonstrated in rats that early treatment with MPH can dramatically alter responsiveness to cocaine during adulthood. Specifically, MPH administered during pre-puberty (PND 20-35) but not in

adulthood (PND 60), decreased rather than increased sensitivity to the rewarding effects of cocaine.

The reasons for the discrepancies found in animal studies examining the effects MPH exposure during development might be attributed to differences in the behavioral assays, dose of MPH, length of MPH treatment, and age of treatment onset. This exemplifies the need for more research in order to examine the particular parameters that lead to specific neural and ultimately behavioral outcomes.

A contentious issue in animal studies concerns the method used to administer MPH. Although humans receive MPH orally, in many animal studies MPH is administered by intraperitoneal (ip) injections (e.g., Kuczenski & Segal, 2002; Gerasimov et al., 2000). Gerasimov et al. (2000) examined the locomotor and neurochemical (i.e., NAcc DA) responses to various MPH doses (2, 5, & 10 mg/kg), administered either orally or by ip injections in rats. Five mg/kg of oral MPH and 2.0 mg/kg ip dose of MPH produced similar levels of DA in the NAcc and similar behavioral activation. The authors concluded: "... one could suggest that ip doses of less than 5 mg/kg may be closer to those used clinically" (p.56). An imaging study in healthy human participants by Volkow et al. (2001) showed that therapeutic oral doses of MPH significantly increased DA levels in the striatum, hence demonstrating for the first time, that therapeutic doses in humans do indeed increase DA levels in reward related brain regions.

Because (1) the therapeutic properties of MPH are undoubtedly related to its effects on neural functioning, (2) ip doses between 2 and 5 mg/kg are considered to be clinically relevant, and (3) dosing by ip injections is accurate and convenient, in the present thesis, I pretreated animals with a 2.5 mg/kg ip dose of MPH.

The purpose of the first experiment was to explore the effects of early exposure to MPH in a typical strain of rat, the Sprague Dawley, on the rewarding properties of cocaine later in adulthood. The rewarding effects of cocaine were assessed using place conditioning.

Method

Subjects and rearing conditions

Ninety-six male Sprague Dawley rats (Charles River, St-Constant, QC) were obtained at either PND 21 (weaning; n= 48) or PND 53 and housed in a temperature and humidity (~22° C, 40-45%) controlled animal facility. Rats were housed in pairs in clear Plexiglass shoebox cages (43.2 (l) × 20.3 (w) × 21.6 cm (h)) with wood shavings lining the floor. Animals had *ad libitum* access to food and water at all times except during testing. Rat cages were cleaned twice a week by facility employees. Enrichment was provided by cardboard rolls and shredded paper that were regularly replaced on cleaning days. The lighting schedule consisted of a 12-h light/ dark cycle (lights on at 08:00). Animals were pretreated and tested during the light phase of the cycle. After an acclimation period of 7 days during which animals were handled, weighed, and numbered, the

pretreatment phase of the experiment began. All experimental procedures were approved by the Animal Care Committee of Concordia University, in accordance with the guidelines of the Canadian Council on Animal Care.

Apparatus

Place conditioning was conducted in automated three-compartment PVC plastic rectangular boxes (Med Associates, Georgia, VT: ENV-013). The overall inside dimensions were 68 cm (l) x 21 cm (w) x 21 cm (h). The apparatus was comprised of distinct compartments that were visually and texturally dissimilar (see figure 1). The middle neutral chamber was gray and was 12 cm long with a smooth PVC floor that could be removed for easy cleaning. The two conditioning chambers were 28 cm long and had different visual and tactile cues (one chamber was black with a stainless steel grid floor and the other was white with a stainless steel mesh floor) that were balanced such that no side preference was exhibited before conditioning. Stainless steel waste pans containing beta chip were provided beneath the grid floors of the two conditioning zones. Each chamber had a hinged clear polycarbonate lid for loading an animal and a ceiling light with a manual dimmer for titrating the natural luminance preferences of the animal. Six photobeam detectors spaced 5 cm apart were positioned across the white and black zones 1.25 cm from the end wall. Three photobeams spaced 4.75 cm apart were positioned across the gray zone. These 15 photobeams made up the computerized system that was run with MED-PC IV software and made it possible to record the time spent in each compartment. Removable guillotine doors controlled the access to the three chambers.

Drug Administration

Methylphenidate hydrochloride (Medisca, Québec, Canada) and cocaine hydrochloride (Medisca, Québec, Canada) were dissolved in 0.9% isotonic saline and injected intraperitoneally. A clinically relevant dose (2.5 mg/kg, ip) of MPH was used during the pretreatment phase (Brandon, Marinelli, Baker, & White, 2001; Gerasimov et al., 2000). During the conditioning phase, four different doses (1.0, 5.0, 10.0, 20.0 mg/kg) of cocaine were used in order to construct a dose-response curve. All doses are expressed as the salt.

Procedure

Pre-Treatment Phase. During the pre-treatment phase, 48 rats (24 peripubertal; 24 adult) were injected once daily with MPH (2.5 mg/kg, ip) and 48 rats (24 peripubertal; 24 adult) were injected with 0.9% saline (1 ml/kg, ip). All injections were administered in the animal colony (home) approximately 5-hours into the light cycle (i.e. around 1:00 pm).

Conditioning Phase. Place conditioning to cocaine was conducted on 48 (24 peripubertal; 24 adult) MPH- and 48 (24 peripubertal; 24 adult) saline-pretreated animals, starting 25 days after the end of the pretreatment phase (PND 63 for peripubertal rats; PND 95 for adult rats). Testing lasted for 6 days and consisted of three phases: preconditioning, conditioning and post-conditioning test (see Figure 2). On the first day (preconditioning), rats were allowed to move freely throughout all three compartments for 30 min and the time spent in each compartment was monitored. For the next four conditioning

days, rats were exposed to once-daily conditioning sessions. They were randomly assigned to receive cocaine pairings with one of the two side compartments and saline pairings with the other compartment in a counterbalanced fashion. On the second and fourth days, they received cocaine (1.0, 5.0, 10.0, or 20.0 mg/kg, i.p.; $n = 6$ per dose) and were confined to one compartment for 30 min. On alternate days, they received saline in the other side compartment. The post-conditioning test was conducted on the 6th day when rats in a drug-free state were allowed to move freely between the three compartments for 30 min, and the amount of time spent in each was recorded.

Statistics

All statistical analyses were conducted using SPSS (version 11.02; SPSS, Chicago, IL) for Mac OS X (Apple Computers, Cupertino, CA). Descriptive statistics were conducted to assess the presence of outliers, and to verify the normality of the distributions. There was no skewness or kurtosis in the data hence no transformations were required. All data were analyzed using ANOVA. Any significant interactions and main effects were followed up by Sidak pairwise comparisons to isolate the source of the significance and to control for familywise error. Furthermore, for all significant ANOVAs, partial eta-squared (η_p^2) are reported as estimates of effect size. The criterion for significance was set at $p < .05$.

Pre-Conditioning. The mean time spent in each chamber was used as the dependent variable. The independent variables consisted of pretreatment,

cocaine dose, and chamber. Separate $2 \times 4 \times 3$ mixed-factorial ANOVAs were conducted for each age group (Peripubertal/ Adult) on the amount of time spent prior to conditioning in the Paired, Neutral, and Unpaired chambers in order to verify if the apparatus was unbiased. The between subject variables were PRETREATMENT (MPH/SAL) and COCAINE DOSE (1, 5, 10, & 20 mg/kg), whereas the within subject variable was CHAMBER (Paired, Neutral, & Unpaired). Please note that the COCAINE DOSE and CHAMBER variables simply represent groups that were conditioned afterward using particular doses in either the black or the white chambers, and hence do not correspond to any manipulation per se during this phase of the experiment.

Post-Conditioning. Place conditioning data are expressed as the difference in time spent in the cocaine- versus the saline-paired compartments before and after conditioning. These mean difference scores were used as the dependent variable for the analysis of all post-conditioning data. The independent variables consisted of pretreatment, cocaine dose, and time. Separate $2 \times 4 \times 2$ mixed-factorial ANOVAs were conducted for the peripubertal and adult treated groups. The between subject variables were PRETREATMENT (MPH/SAL) and COCAINE DOSE (1, 5, 10, & 20 mg/kg), whereas the within subject variable was TIME (before vs. after conditioning).

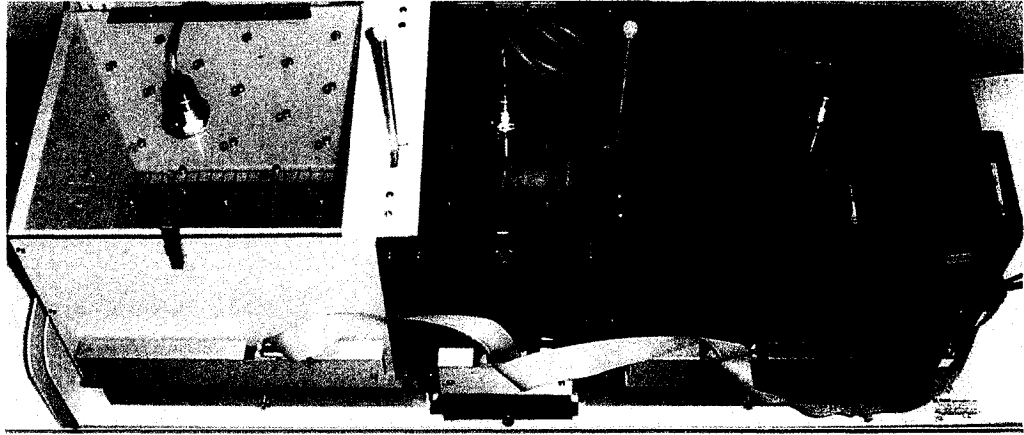


Figure 1. A pictorial representation of the automated three-compartment apparatus used to assess conditioned place preference.

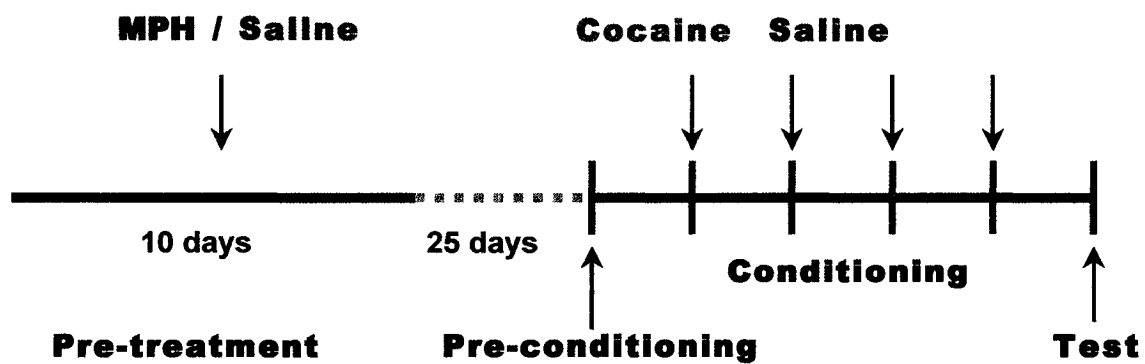


Figure 2. Graphical representation of the procedural timeline used in Experiments 1 and 2.

Results

Pre-Conditioning

Peripubertal pretreated group. As can be seen in Figure 3, animals spent an equal amount of time in the PAIRED ($M = 729.65$, $SE = 18.04$) and UNPAIRED ($M = 666.67$, $SE = 18.27$) chambers, and less time in the NEUTRAL ($M = 403.68$, $SE = 13.86$) chamber. The mixed-factorial ANOVA revealed a significant effect of CHAMBER, $F(2, 80) = 70.23$, $\eta_p^2 = .64$, $p < .001$. Post-hoc comparisons confirmed that there were no significant differences between the time spent in the PAIRED and UNPAIRED chambers demonstrating that the apparatus was unbiased prior to conditioning. Similar results were found in animals pretreated with MPH during adulthood.

Adult pretreated group. As can be seen in Figure 4, animals spent an equal amount of time in the PAIRED ($M = 771.75$, $SE = 18.19$) and UNPAIRED ($M = 744.03$, $SE = 19.29$) chambers, and significantly less time in the NEUTRAL ($M = 286.31$, $SE = 9.52$) chamber. The mixed-factorial ANOVA revealed a significant effect of CHAMBER, $F(2, 80) = 187.67$, $\eta_p^2 = .82$, $p < .001$. Post-hoc comparisons revealed that there were no significant differences between the time spent in the PAIRED and UNPAIRED chambers, confirming that the apparatus was truly unbiased.

Post-Conditioning

Peripubertal pretreated group. Significant interactions between TIME \times PRETREATMENT, $F(1, 40) = 5.07, \eta_p^2 = .11, p < .001$, and TIME \times COCAINE DOSE, $F(3, 40) = 6.05, \eta_p^2 = .31, p < .001$, were found. As illustrated in Figure 5, post hoc comparisons showed that after conditioning, saline-pretreated rats given 5.0, 10.0, 20.0 mg/kg spent more time ($M = 339.06, SE = 105.52, M = 558.98, SE = 105.52, M = 585.02, SE = 105.52$) in cocaine-associated compartments than they did before ($M = 76.47, SE = 94.22, M = 70.61, SE = 94.92, M = 67.01, SE = 94.22$). No significant results were found for the lowest dose of cocaine tested (1.0 mg/kg). In the MPH-pretreated rats, however, 1.0, 5.0, or 10.0 mg/kg cocaine failed to alter the time spent in drug-paired compartment. Only the rats pretreated with 20.0 mg/kg of cocaine established a place preference (before: $M = 124.56, SE = 94.92$; after: $M = 492.21, SE = 105.52$).

Adult pretreated group. A significant interaction between TIME \times COCAINE DOSE, $F(3, 40) = 5.87, \eta_p^2 = .31, p < .01$, was found. As illustrated in Figure 6, post hoc comparisons showed that after conditioning, regardless of pretreatment, rats given 5.0, 10.0, 20.0 mg/kg spent more time ($M = 545.34, SE = 88.15, M = 656.07, SE = 88.15, M = 611.87, SE = 88.15$) in cocaine-associated compartments than they did before ($M = 71.48, SE = 72.57, M = 68.98, SE = 72.57, M = -4.45, SE = 72.57$). No significant results were found for the lowest dose of cocaine tested (1.0 mg/kg).

Figure 3. Mean (\pm SEM) time spent in either the Paired, Neutral, or Unpaired chamber prior to conditioning (n = 6 rats/grp) in MPH and SAL peripubertally pretreated rats.

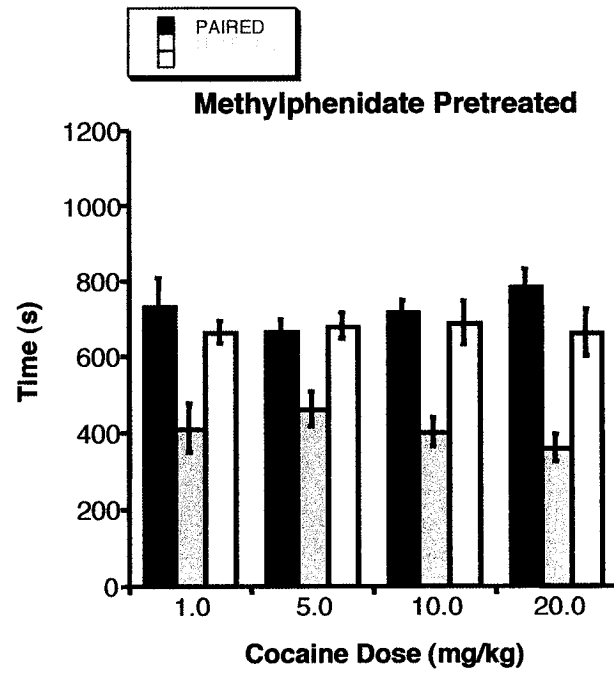
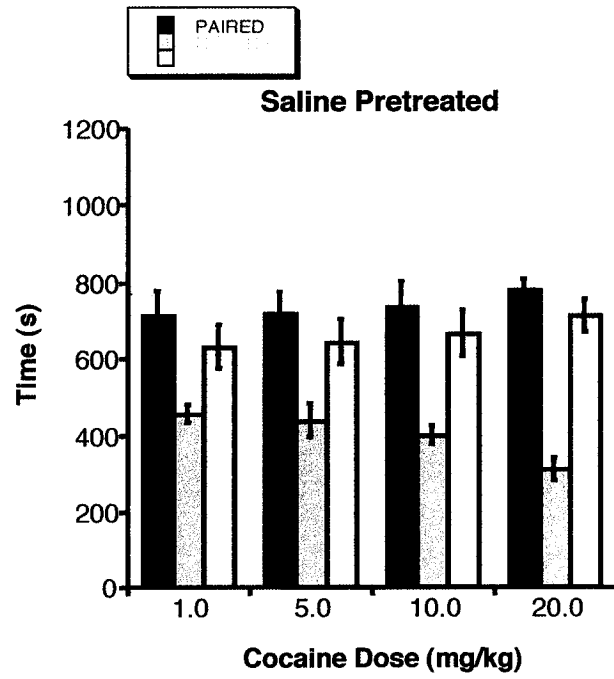


Figure 4. Mean (\pm SEM) time spent in either the Paired, Neutral, or Unpaired chamber prior to conditioning (n = 6 rats/ grp) in adult MPH and SAL pretreated rats.

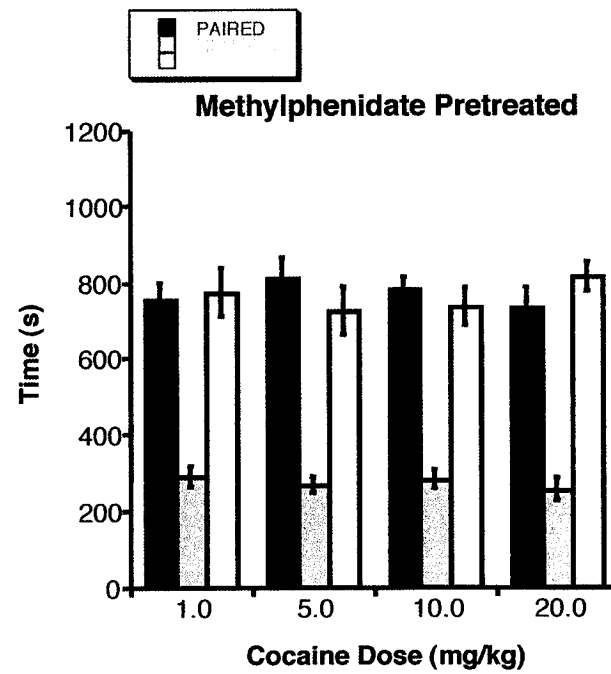
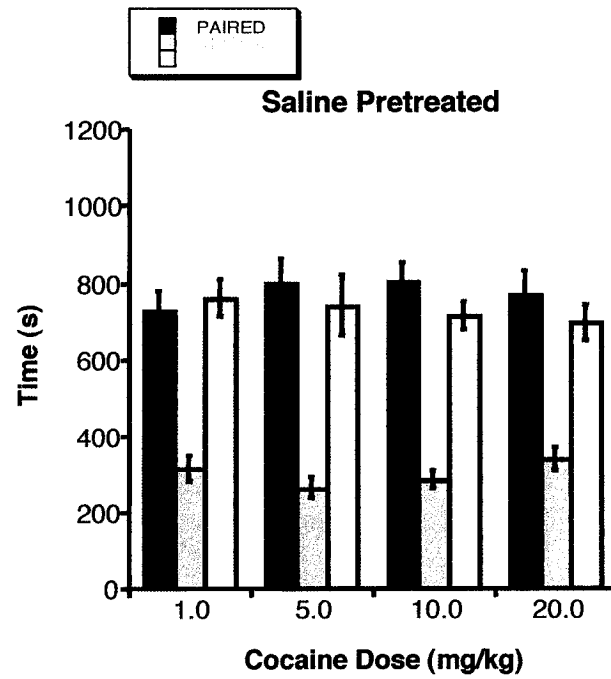


Figure 5. Effects of peripubertal MPH pretreatment on cocaine place conditioning during adulthood. Place preference (mean \pm S.E.M) is expressed as the change in time spent in the drug side minus time spent in the saline side before (white bars) and after (red bars) conditioning. Data from saline- and MPH-pretreated rats are shown on the top and bottom panels, respectively.

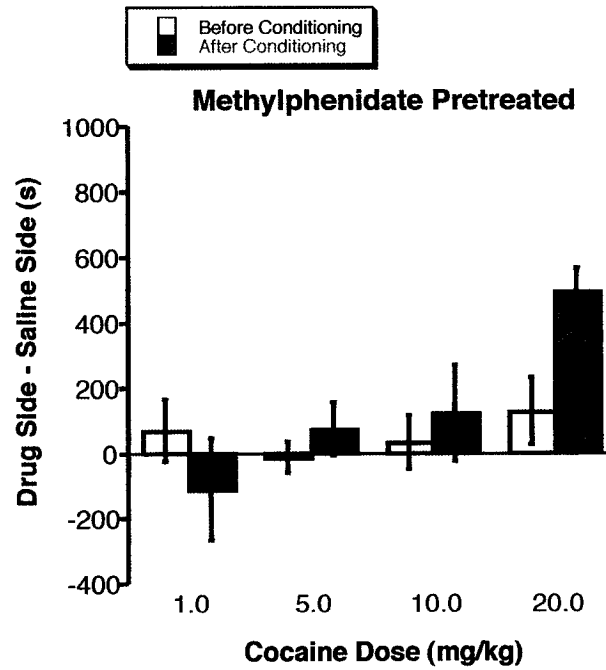
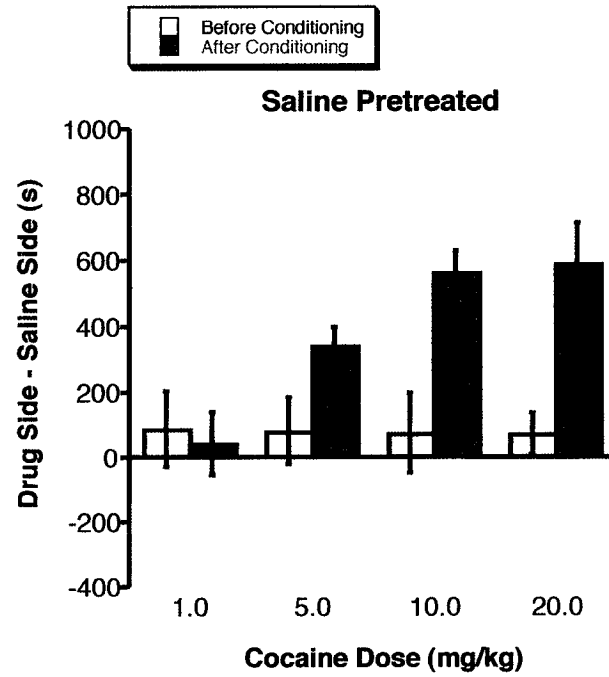
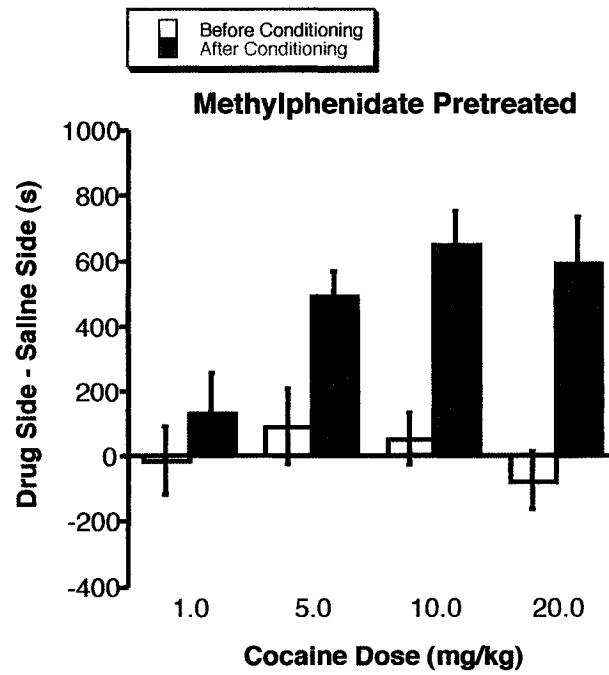
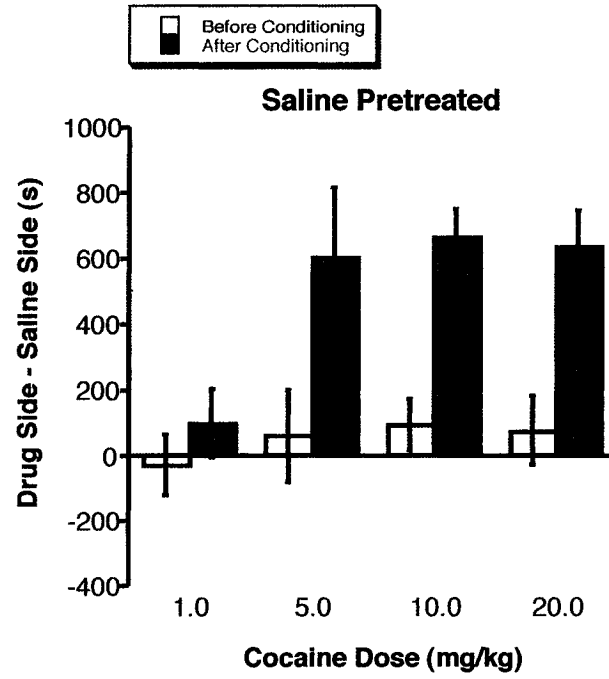


Figure 6. Effects of adult MPH pretreatment on cocaine place conditioning later in adulthood. Place preference (mean \pm S.E.M) is expressed as the change in time spent in the drug side minus time spent in the saline side before (white bars) and after (red bars) conditioning. Data from saline- and MPH-pretreated rats are shown on the top and bottom panels, respectively.



Experiment 2

Despite being the most common disorder of childhood, the etiology of ADHD remains largely unknown. Furthermore, because ADHD is considered a heterogeneous psychiatric disorder comprised of various behavioral and cognitive impairments, some researchers question whether it can be considered a discrete disorder (Davids, Zhang, Tarazi, & Baldessarini, 2003). Despite the heterogeneous nature of ADHD and the fact that there is no definite approach in diagnosing the disorder, it is characterized by certain core features, including inattention, impulsivity, and hyperactivity, that typically appear during early childhood (Barkley, 1998; Barkley, Fischer, Smallish, & Fletcher, 2004; Castellanos et al., 1994; Fischer & Barkley, 2003).

As is the case with many neuropsychiatric disorders, much of what is known about ADHD has come from animal studies that try to model the disorder and its symptomatology (Adriani, Caprioli, Granstrem, Carli, & Laviola, 2003; Davids, Zhang, Tarazi, & Baldessarini, 2003; Kuczenski & Segal, 2005; Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005). Based on the results of such studies, much consideration has been given to the hypothesis that core symptoms may be related to abnormal functioning within the monoaminergic brain system, and particularly, its DAergic component (Arnsten, 2006; Carey et al., 1998; Castellanos et al., 1994; Solanto, 1998, 2002; Viggiano, Vallone, & Sadile, 2004). DAergic dysfunction in human ADHD patients has been confirmed by brain imaging studies. For example, after reviewing numerous imaging studies conducted in ADHD patients and healthy controls, Krause, Dresel, Krause, la

Fougere, & Ackenheil (2003) concluded that the levels of DAT in striatal regions are significantly higher in people with ADHD compared to controls. The functional consequence of having higher DAT levels are predicted to be related to lower extracellular DA levels by virtue of an enhanced clearance capacity. DA enhancing agents, such as MPH and amphetamine, may thus alleviate the core symptoms associated with the disorder by correcting DA hypofunction (Arnsten, 2006; Berridge et al., 2006; Viggiano, Vallone, & Sadile, 2004; Volkow, Fowler, Wang, Ding, & Gatley, 2002). Because numerous studies allude to a dysfunctional DA system in ADHD, one could question the validity of studying the long-term effects of MPH in typical subjects (Hyman, 2003; Shen & Choong, 2006). Thus, researchers have tried to establish animal models of ADHD.

A commonly used rodent model of ADHD is the spontaneously hypertensive rat (SHR). While these rats were initially bred for studies relating to hypertension, they were found serendipitously to express locomotor hyperactivity (Moser, Moser, Wultz, & Sagvolden, 1988; Wultz, Sagvolden, Moser, & Moser, 1990). In later studies by various groups, SHR rats were also found to express other core symptoms of ADHD such as a hypofunctional DA system and impulsivity (Oades et al., 2005; Russell, 2002; Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005). Further validation for the SHR as an animal model of ADHD comes from the fact that most of the ADHD-like symptoms expressed by these rats, and similar to human patients, are alleviated by stimulant drugs such as MPH (Davids, Zhang, Tarazi, & Baldessarini, 2003; Mook & Neuringer, 1994; Myers, Musty, & Hendley, 1982; Sagvolden et al., 1992)

In the first experiment reported in this thesis I investigated the effects of MPH exposure in a typical strain of rats. The present experiment examines the effects of early treatment with MPH in SHR rats that display several core features of ADHD and have been reasonably well validated as an animal model of ADHD.

Method

Subjects and rearing conditions

Forty-eight male SHR rats (Charles River, St-Constant, QC) were obtained at postnatal day (PND) 28 (weaning) and housed in a temperature and humidity (~22° C, 40-45%) controlled animal facility. Other than using a different strain of rats, and only the peripubertal cohort, all other procedures were exactly as in experiment 1. Please refer to Figure 2 for a summary.

Results

Pre-Conditioning

The mixed-factorial ANOVA revealed a significant interaction between CHAMBER \times COCAINE DOSE, $F(6, 80) = 3.56$, $\eta_p^2 = .21$, $p < .01$. Post-hoc comparisons revealed that within each dose (1.0, 5.0, 10.0, 20.0 mg/kg) animals spent an equal amount of time in the PAIRED (1.0 mg: $M = 698.36$, $SE = 24.79$; 5.0: $M = 688.84$, $SE = 24.79$; 10.0: $M = 702.30$, $SE = 24.79$; 20.0: $M = 628.37$, $SE = 24.79$) and UNPAIRED (1.0 mg: $M = 673.84$, $SE = 27.72$; 5.0: $M = 759.27$, $SE = 27.72$; 10.0: $M = 716.58$, $SE = 27.72$; 20.0: $M = 660.75$, $SE = 27.72$) chambers, and significantly less time in the NEUTRAL (1.0 mg: $M = 427.80$, $SE = 28.63$; 5.0: $M = 355.97$, $SE = 28.63$; 10.0: $M = 381.12$, $SE = 28.63$; 20.0: $M = 510.89$, $SE = 28.63$) chamber. This demonstrates that the apparatus was unbiased prior to conditioning (See Figure 7).

Post-Conditioning

A $2 \times 4 \times 2$ mixed-factorial ANOVA was conducted on the mean difference of time spent in the cocaine versus the saline-paired chambers (as described for experiment 1). The between subject variables were PRETREATMENT (MPH/SAL) and COCAINE DOSE (1, 5, 10, & 20 mg/kg), whereas the within subject variable was TIME (before vs. after conditioning). Significant interactions between TIME \times PRETREATMENT, $F(1, 40) = 6.29$, $\eta_p^2 = .14$, $p < .01$, and TIME \times COCAINE DOSE, $F(3, 40) = 5.46$, $\eta_p^2 = .29$, $p < .01$, were

found. As illustrated in Figure 8, post hoc comparisons showed that after conditioning, saline-pretreated rats given 5.0, 10.0, 20.0 mg/kg spent more time ($M = 466.26, SE = 96.26, M = 457.82, SE = 96.26, M = 413.19, SE = 96.26$) in cocaine-associated compartments than they did before conditioning ($M = -80.46, SE = 62.32, M = -51.35, SE = 62.32, M = -25.29, SE = 62.32$). No significant results were found for the lowest dose of cocaine tested (1.0 mg/kg). In the MPH-pretreated rats, however, 1.0, 5.0, or 10.0 mg/kg cocaine failed to alter the time spent in drug-paired compartment. Only in the case of the rats pretreated with 20.0 mg/kg cocaine established a place preference (before: $M = -39.48, SE = 62.32$; after: $M = 400.52, SE = 96.26$).

Figure 7. Mean (\pm SEM) time spent in either the Paired, Neutral, or Unpaired chamber prior to conditioning (n = 6 rats/ grp) in MPH and SAL peripubertally pretreated SHR rats.

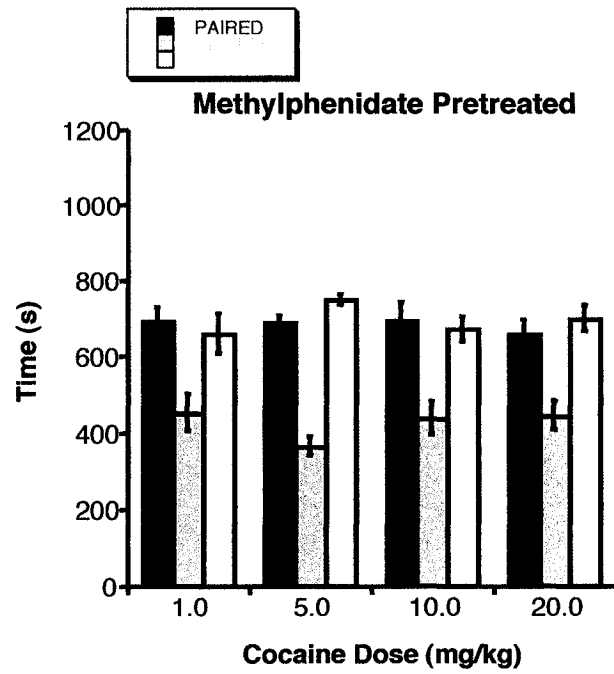
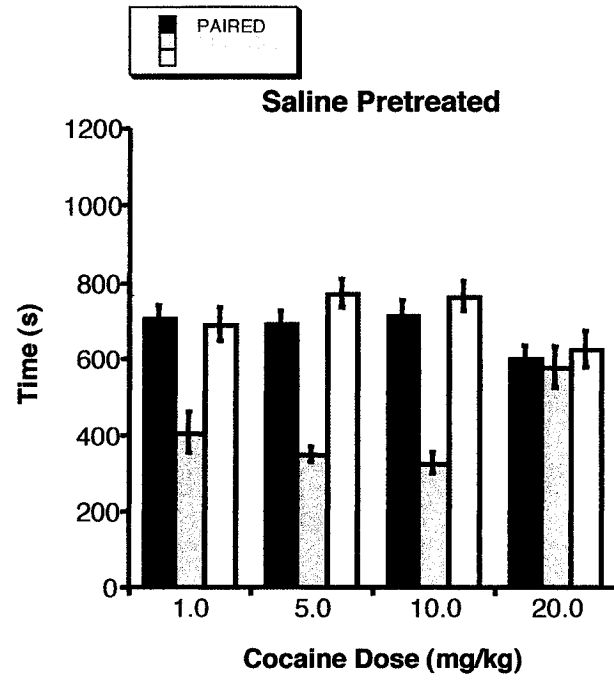
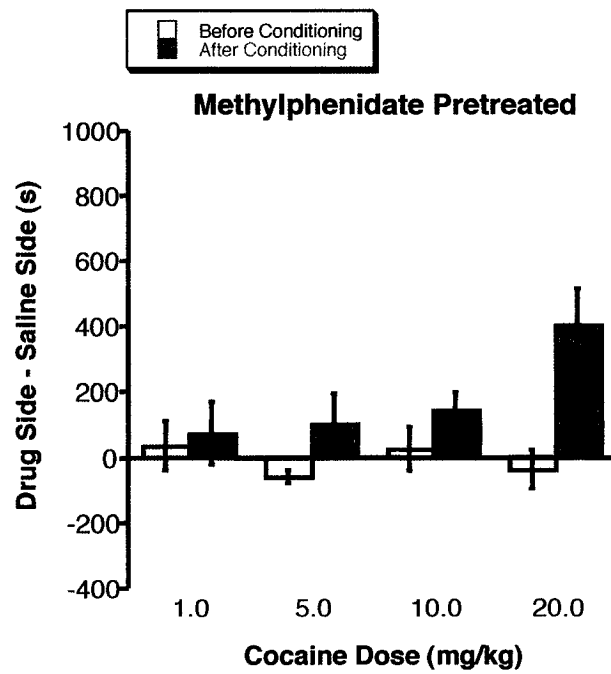
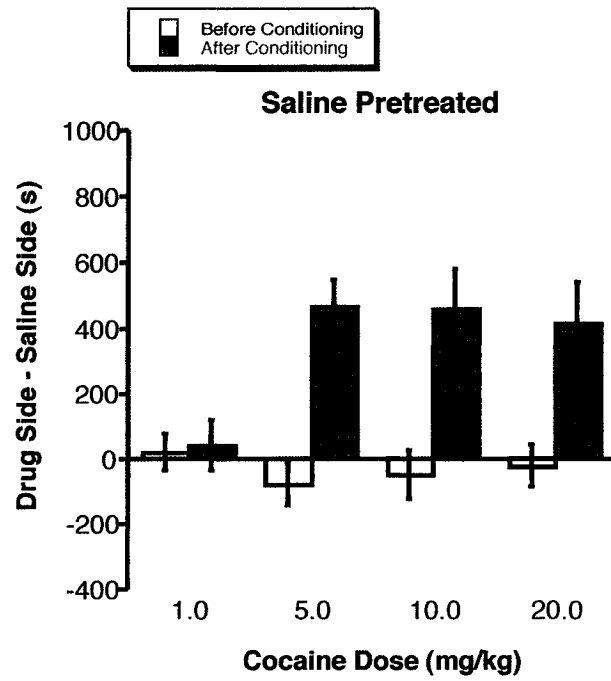


Figure 8. Effects of peripubertal MPH pretreatment on cocaine place conditioning during adulthood. Place preference (mean \pm S.E.M) is expressed as the change in time spent in the drug side minus time spent in the saline side before (white bars) and after (red bars) conditioning. Data from saline- and MPH-pretreated rats are shown on the top and bottom panels, respectively.



General Discussion

Children and adolescents are increasingly prescribed MPH for the treatment of ADHD. Controversy surrounds this practice since repeated exposure to stimulant drugs causes enduring changes in the brain that along with genetic and environmental factors are thought to contribute to addiction (Badiani & Robinson, 2004; T. E. Robinson & Berridge, 2001). In spite of its popularity and success in attenuating ADHD symptomatology, relatively little is known about the consequences of MPH on future drug use and associated risks for addiction. It is also unclear whether the effects of MPH vary between individuals with or without ADHD symptomatology. This last matter is important in view of the recent upsurge in stimulant treatment of children that, in some cases, may be misdiagnosed, and inadvertently medicated with MPH (Carlezon & Konradi, 2004). The experiments reported in this thesis were aimed at addressing both of these issues.

The results of the first experiment demonstrated an age-dependent effect of MPH exposure on the rewarding properties of cocaine in adulthood. Specifically, it was found that MPH exposure during the peripubertal period significantly reduced the rewarding properties of low and moderate doses of cocaine as measured by place conditioning. No such effects were found in MPH pretreated adult rats, which did not differ from saline pretreated controls. This suggests that effects of drugs on the functioning of the brain may not be identical throughout ontogeny.

Since four increasing doses of cocaine were used during the conditioning phases of the experiments, the results can be discussed in terms of DRCs. Upon examination of the amount of time animals spent in the drug-paired compartments following conditioning, regardless of rat strain, it can be seen that a rightward shift in the DRC occurred in the adolescent MPH pretreated rats. That is, a higher dose of cocaine (20mg/kg, i.p.) was needed in these animals to establish robust place conditioning in adulthood, whereas no such effect was present in either the adult MPH pretreated or any of the control animals. Rightward shifts in DRCs are indicative of tolerance-like adaptations, which following repeated drug-exposure render lower doses of the drug less efficient (Lett, 1989; Stewart & Badiani, 1993). The fact that these rightward shifts were manifested following a protracted drug-free period (i.e., development into adulthood) is representative of long-lasting alterations in brain reward circuitry as a consequence of developmental MPH exposure.

Another finding worthy of note was the observation that MPH given during the peripubertal period reduced the rewarding properties of cocaine regardless of whether the exposure occurred in an animal model of ADHD, the SHR rat, or a typical rat strain. The SHR rat has been validated as being one of the best animal models of ADHD (Sagvolden, 2000; Sagvolden, Russell, Aase, Johansen, & Farshbaf, 2005). The results of the second experiment demonstrate that prolonged MPH exposure early in development does not differentially affect this strain of rats. While the effectiveness of MPH treatment may be sensitive to the severity of ADHD symptomatology (Vaidya et al., 1998; Vaidya et al., 2005), it seems that MPH affects the neural circuitry responsible for processing the

incentive motivational effects of drugs similarly in both typical and atypical subjects. Although more research is needed, these results, by extrapolation, suggest that drug-related circuitry may not be directly involved in the symptoms of ADHD.

Seen from another perspective, the finding that MPH exerts similar effects on drug-related circuitry in both typical and SHR rats, seems to parallel the long-standing observation that in humans, the ability of MPH to enhance cognitive functioning and attention, as well as reduce restlessness, is similar in individuals affected with ADHD and those not affected (Rapoport et al., 1978; Rapoport & Inoff-Germain, 2002).

Based on the massive body of literature pertaining to drug sensitization (Kalivas & Stewart, 1991; Kuczenski, Segal, & Todd, 1997; Lett, 1989; T. E. Robinson & Berridge, 2001; T. E. Robinson & Kolb, 1997; Stewart & Badiani, 1993), the findings presented in this thesis are opposite to what one would expect following repeated exposure to psychostimulants. However, since the tolerance-like effects in the present experiments were observed specifically in the adolescent and not adult MPH pretreated animals, it remains unclear why there would be such an age-dependant effect of drug exposure. That said, it has been reported that the effects, or responses, to drugs vary depending on the age at which they are administered (Bolanos, Glatt, & Jackson, 1998; Spear, 2000). While adolescence is a developmental period often related with heightened sensation-seeking and risk-taking behaviors that can predispose to drug use and abuse (Chambers, Taylor, & Potenza, 2003; Laviola et al., 2004; Tirelli, Laviola, &

Adriani, 2003), there is evidence suggesting that both adolescent animals and humans have blunted responses to stimulant drugs (Bolanos, Glatt, & Jackson, 1998; Spear, 2000). In light of this evidence, more research is needed to fully quantify the parameters, including ontogenic-dependent variations in drug responses, that can affect the development of drug induced neural alterations such as tolerance and sensitization.

In spite of the need for more research into the effects of developmental exposure to psychostimulant drugs such as MPH, the results of the present experiments fit well with several recent reports. Using a similar experimental procedure, Andersen, Arvanitogiannis, Pliakas, LeBlanc, and Carlezon (2002) found using CPP, that MPH exposure during adolescence reduced the rewarding properties of cocaine in adulthood. Similarly, using intracranial self-stimulation, an operant paradigm where animals respond for rewarding electrical brain stimulation, Mague, Andersen, and Carlezon (2005) found that developmental MPH exposure diminished the reward-potentiating effects of cocaine.

While all of these studies pertain specifically to the rewarding properties of cocaine following developmental MPH exposure, other studies have shown similar effects with natural rewards. In an interesting study by Bolanos, Barrot, Berton, Wallace-Black, and Nestler (2003), it was found that MPH treatment during the periadolescent period similarly reduced sensitivity to the rewarding properties of sucrose and sexual behavior.

Although it could be suggested that the specific pharmacological properties of MPH could explain why MPH exposure decreases the rewarding properties of both drugs such as cocaine, and natural rewards such as sucrose and sex, such a possibility is improbable. First, there is no robust evidence suggesting that MPH's actions are much different from other stimulants. Actually there is an abundance of evidence suggesting important similarities between MPH and other stimulant drugs such as cocaine (Gatley et al., 1999; Volkow et al., 1995; Volkow et al., 1999). Second, sensitization and cross-sensitization of both the incentive properties and behavioral activating effects of MPH have been reported (Crawford, McDougall, Meier, Collins, & Watson, 1998; Gaytan, Swann, & Dafny, 2002; Meririnne, Kankaanpaa, & Seppala, 2001; Schenk & Izenwasser, 2002).

Even more convincing evidence comes from a recent study by Carlezon, Mague, and Andersen (2003) where adolescent rats were pretreated twice daily from PND 20-35 with either MPH (2.0mg/kg, i.p) or cocaine (15mg/kg, i.p.). Following this pretreatment, the rewarding properties of cocaine, using CPP, were assessed in adulthood. Remarkably, both the cocaine and MPH pretreated rats showed indistinguishable reductions in the rewarding properties of moderate to high doses of cocaine. These results are interesting since they clearly demonstrate that both MPH and cocaine given during a specific developmental period can decrease rather than increase the rewarding properties of cocaine. In addition, on the basis that MPH pretreatment during adulthood did not alter the rewarding properties of cocaine in the first experiment, it seems reasonable to

conclude that it is the age at which animals are exposed to the treatment and not the actions of MPH per se that causes the observed changes.

In summary, the results from recent studies combined with the results of the present experiments suggest that tolerance as opposed to sensitization develops following adolescent MPH exposure. Whereas sensitization and its related neuroadaptations are believed to be intrinsically linked with addiction (T. E. Robinson & Berridge, 2001, 2003), the current results, demonstrating tolerance-like effects following adolescent MPH pretreatment, would be related to a decreased propensity for addiction. Such a conclusion corresponds surprisingly well with reports from human studies indicating that MPH treatment in ADHD actually reduces the risk for substance abuse later in adulthood (Biederman, 2003; Biederman, Wilens, Mick, Spencer, & Faraone, 1999; Mannuzza, Klein, & Moulton, 2003).

In as much as the present results show that periadolescent MPH exposure alters the rewarding properties of cocaine later in adulthood, it can be inferred from the longevity of these effects, that enduring changes in the neural substrates involved in reward have been altered. While the exact mechanisms involved in the modulatory effects of MPH during adolescence on future reward-related phenomena remain unknown at present, some intuitive suggestions can be put forth.

Much evidence supports the notion that drugs as well as natural rewards including sex and food, exert their incentive effects by increasing DA levels in

the mesolimbic DA system, and in particular in the NAcc (Bassareo & Di Chiara, 1999; Di Chiara & Imperato, 1988; Léyton et al., 2002; Pfaus et al., 1990).

Accordingly, higher drug-induced DA release is related to increases in the rewarding properties of drugs, which behaviorally can be defined as augmented drug-seeking behavior (Piazza & Le Moal, 1996). Conversely, reduced DA release is associated with decreases in the rewarding properties of drugs (Wise, 1996).

Based on the idea that NAcc DA levels are implicated in the incentive properties of drugs, our group has investigated, through collaborative work, whether the decreases in the rewarding properties of cocaine in adolescent MPH pretreated rats are coupled with differences in extracellular DA levels in the NAcc. Using the same MPH/SAL pretreatment regimen described in the present thesis, adolescent SHR rats were then challenged, in adulthood, with a moderate dose of cocaine (10mg/kg, ip) while NAcc DA was being sampled. Surprisingly, no significant differences were found in cocaine-induced DA release between the MPH and saline pretreated animals (Augustyniak, Kourrich, Rezazadeh, Stewart, & Arvanitogiannis, 2006). These results demonstrate that alterations in the rewarding properties of cocaine as a function of MPH treatment during the peripubertal period are not explained by alterations of extracellular DA levels in the NAcc.

Stimulant drugs exert their rewarding effects by activating not only the mesolimbic DA system projecting to the NAcc but also the mesocortical DA system projecting to the PFC (Taber & Fibiger, 1995; Tzschentke & Schmidt, 2000). Mesocortical and mesolimbic DA neurons have distinct anatomical and

functional characteristics. For instance, mesocortical, but not mesolimbic DA projections are directly innervated by cortical glutamatergic neurons (Carr, O'Donnell, Card, & Sesack, 1999; Carr & Sesack, 2000). In addition, results from pharmacological and lesion experiments have shown that the function of mesolimbic and mesocortical DA systems in adult rats can be regulated in opposite directions (Banks & Gratton, 1995; Sorg, Davidson, Kalivas, & Prasad, 1997). These studies have also shown that enhanced DA neurotransmission in the PFC attenuates DA activity in the NAcc. Accordingly, future research will be needed to investigate the potential of MPH treatment to alter PFC DA release in response to various rewards, and whether any of these differences are dependent upon the age of MPH exposure.

In addition to altering DA release in various brain regions, drugs exert their long-term effects on neural circuitry by regulating the types and amounts of proteins present in DA neurons and their targets (Berke, Paletzki, Aronson, Hyman, & Gerfen, 1998; Chao & Nestler, 2004). Drug-induced regulation of protein levels is likely to involve changes in gene transcription achieved by the regulation of transcription factors such as cAMP response element-binding protein (CREB) (Carlezon et al., 1998; Nestler, 2001). Pertinent to the present discussion is the finding that elevated CREB levels in the NAcc have been related with decreases in the rewarding properties of cocaine (Carlezon & Konradi, 2004; Carlezon et al., 1998). Accordingly, it has been found that repeated MPH exposure during adolescence diminishes the rewarding properties of cocaine, and these reductions were associated with large increases in CREB expression in

the NAcc later in adulthood (Andersen, Arvanitogiannis, Pliakas, LeBlanc, & Carlezon, 2002).

Finally, it should be noted that the mesocorticolimbic DA system does not function in isolation. One important component of the neuronal circuitry engaged by stimulant drugs and known to innervate and directly influence the DA system is the glutamatergic system (Hyman & Malenka, 2001; Kalivas, 2004). In fact, glutamatergic inputs to both the VTA and NAcc arising from various brain regions including the PFC, hippocampus, and basolateral amygdala, have been implicated in the long-lasting effects of stimulant drugs (Brog, Salyapongse, Deutch, & Zahm, 1993; Kelley & Domesick, 1982; Kelley, Domesick, & Nauta, 1982). Particularly, GluR1 and GluR2, two AMPA receptor subunits, have been found to be related with either heightened or decreased responsivity to the rewarding properties of cocaine. That is, elevated levels of GluR1 in the NAcc were found to decrease the rewarding effects of cocaine, whereas elevated GluR2 levels had opposite effects (Kelz et al., 1999). Since glutamate, and in particular its AMPA receptor composites have been implicated in the incentive properties of stimulant drugs, it is plausible to assume that developmental MPH exposure can differentially affect this system. Future research will be needed to address this question.

Conclusions

In summary, the experiments summarized in the present thesis show that periadolescent MPH decreases rather than increases the sensitivity to the rewarding effects of cocaine later in adulthood. In addition, it was found that MPH exposure during this developmental period affects the rewarding properties of cocaine in both an animal model of ADHD and a typical strain of rats. By extrapolation, one could argue that human adolescents treated with MPH would have a lower propensity to abuse drugs by virtue of their diminished incentive properties. Such a conclusion, although it seems to fit with reports from human studies, is precarious. Clearly, diverse sources of data are needed to sketch an accurate portrait of the long-term effects of early developmental exposure to MPH. Although the exact mechanisms involved remain unknown, these findings exemplify the notion that the consequences of prolonged drug exposure are under the influence of many factors, and that ultimately they may not be as clear-cut as was previously thought (Carlezon & Konradi, 2004).

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