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Comparison between Two Methodological Paradigms of Conditioned Place Preference with Methlyphenidate.

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Abstract

The aim of this thesis is to examine the mechanisms of Methylphenidate (MPH) on Conditioned Place Preference (CPP), a behavioral test of reward. The psychostimulant MPH is therapeutically used in the treatment of ADHD, but has been implicated in many pharmacological actions related to drug addiction and is considered to have abuse potential. Past work in our lab and others have shown substantial sex-differences in the neuropharmacological profile of MPH. Here a discussion of the relevant mechanisms of action of MPH and its relationship to neurotrophins and CPP are reviewed. Furthermore, previous work is reviewed and a rationale for two experiments are presented. The study resulted in two experiments conducted earlier this year. Each experiment is presented, examining sex differences in CPP in two different methodological paradigms as well as the effects of MPH on levels of the dopamine transporter (DAT) in striatal and accumbal neurons.

Introduction

Methylphenidate (MPH) is a psychostimulant sold under the trade-name Ritalin. This class of drugs also includes cocaine, amphetamine, and methamphetamine. MPH is clinically prescribed for the treatment of ADHD and narcolepsy. It is one of the most commonly prescribed medications for ADHD. An estimated 6% of school-aged children are prescribed with either MPH or amphetamine, with MPH representing nearly 90% of psychostimulant prescriptions (Volkow and Swanson 2003, Zito et al. 2000). In the 1990s, there was a five-fold increase in the number of children who were prescribed psychostimulants leading to concern about the long-term effects of psychostimulant prescription among children (Mayes et al. 2008).

At present no biomedical lab test exists for diagnosis of ADHD. Diagnosis historically has been based on the observation of behavioral symptoms which are common in children

(Mayes et al. 2008, Tripp and Wickens 2009). Furthermore, there is a lack of homogeneity in many study populations leading some researchers to question the existence of a unified etiology (Tripp and Wickens 2009). MPH is clinically effective and safe for ADHD symptoms; however, rising rates of prescription are cause for concern. Furthermore, due to ambiguity of symptoms and diagnostic and treatment styles of physicians, ADHD has been severely over-diagnosed and under-diagnosed in different geographic regions (Mayes et al. 2008).

Though there is still extensive debate, salient features of ADHD are widely agreed upon. Broad deficits in executive function and altered reward and motivation have been thought to underlie many symptoms of ADHD (Barkley 1997, Tripp and Wickens 2009) Clinical doses of MPH may serve to balance neurotransmitter release in the prefrontal cortex (PFC) thus relieving executive function deficits (Berridge and Devilbiss 2006). An altered response to reinforcement has also been demonstrated in children with ADHD and several models have been proposed in which altered sensitivity to reinforcement is a cardinal symptom (Luman et al. 2005). Stronger preference for immediate reinforcement over delayed reinforcement explain many of the symptoms of ADHD. Psychostimulants such as MPH have been shown to selectively control the efficacy of conditioned reinforcement (Hill 1970, Robbins 1975, Tripp and Wickens 2009). However, research has shown that MPH has a similar neuropharmacological profile to that of commonly abused drugs such as cocaine and amphetamine. This combined with rising rates of prescription has raised concerns of the abuse potential of MPH (Volkow et al. 1999) The rising rates of prescription of adolescents require greater study into the long term effects of MPH in adolescents. Earlier ages of prescription and longer treatment durations may affect developmental processes into adulthood, leading to structural changes and long-term behavioral changes (Scherer et al. 2010, Zito et al. 2000). Thus a primary goal of the research presented here

is to investigate possible mechanisms of action that may elucidate the effects of MPH on abuse potential and developmental outcome.

Abuse of MPH has been a cause of concern in recent years due to its availability. In one study, about 20% of patients ages 12-18 in an addiction center were determined to have misused MPH for recreational purposes. About 5% were diagnosed as MPH abusers. Despite lower rates of abuse relative to other psychostimulants such as cocaine, MPH still shows significant abuse potential (Williams et al. 2004). In 2002 it was found that approximately 7.3 million persons in the United States aged 12 and older had misused ADHD medications. These abused drugs were usually psychostimulants prescribed for ADHD, with MPH being the most popular (Kroutil et al. 2006) The speed of change in blood-serum levels which can be attained through intravenous or intranasal use may increase potential for addiction (Volkow and Swanson 2003). These routes of administration are more common for non-medical usage of stimulants and there is evidence that MPH may be more dangerous than cocaine and amphetamine when used in this way (Parran and Jasinski 1991).

Rates of prescriptions for MPH in the United States and Canada have risen dramatically since the early 1990s (Poulin et al. 2001). However, the long-term effects of MPH have not been studied sufficiently. There is also some evidence that chronic usage of MPH during adolescence may have lasting behavioral consequences. Chronic MPH exposure during development has been linked to decreased sensitivity to rewarding stimuli and enhanced sensitivity to aversive situations (Bolaños et al. 2003). Thus with rising rates of prescription, it is important to further study the potential neurological changes resulting from accepted use and abuse of MPH.

Methylphenidate Mechanism and Neurotrophic Effects

The primary effects of MPH are due to inhibition of the dopamine transporter (DAT) as well as the norepinephrine transporter (NET). Binding to these transporters serves to block reuptake of post synaptic dopamine and norepinephrine leaving abnormally high extracellular levels of neurotransmitters in the synaptic cleft.

DAT is a critical transporter protein that removes extracellular DA released from the synapse, thereby lowering the levels of available DA. Furthermore, DAT levels may vary in different brain regions (Yang et al. 2007). Due to blockage of the DAT, extracellular DA levels are thought to accumulate in cellular medium. Heightened DA availability increases binding to cellular DA receptors. Furthermore, extracellular DA concentrations have been shown to be significantly higher in the human brain after a therapeutic dose of MPH was given. Researchers found that extracellular DA was increased in the striatum, a key brain area for reward activity (Volkow et al. 2001). Further elucidating the DAT role in the therapeutic efficacy of MPH is research showing that patients with ADHD have significantly elevated levels of striatal DAT (Krause et al. 2000, Dougherty et al., 1999). These findings may be particularly relevant to MPH's therapeutic efficacy because DA levels in the striatum have been shown to affect the signal-to-noise ratio of striatal cells (Kiyatkin and Rebec 1996). Patients with ADHD may therefore have increased levels of extracellular DA occupying a higher percentage of DAT when taking methylphenidate and thus have reduced background cell firing rates and enhanced synchronous goal-seeking neuronal activity (Volkow et al. 2001). In a past study DA increase was significant in all subjects, but highly variable. In particular, it is important to note that all subjects were healthy controls although there is little reason to think that oral MPH would act differently on DAT in patients with ADHD (Volkow et al. 2001). However, this is relevant to our

experimental model that focuses more closely on the abuse potential of MPH than on therapeutic action for ADHD.

The pharmacokinetics of MPH and route of administration may be important to explaining its abuse potential. A therapeutic dose of oral MPH is generally 0.25-1 mg/kg. This dosage has been shown to potently block 50%-75% of DAT receptors in humans (Volkow et al. 1998). Oral MPH reaches peak concentrations in the brain after 60 minutes, however intravenous MPH has been shown to reach peak concentrations within 8-10 minutes (Volkow et al. 1995). Perhaps most importantly the affinity with which MPH blocks DAT has been shown to have a similar psychopharmacological potency to that of cocaine (Volkow et al. 1998). Supporting this research, it has been shown that there is a significant relationship between self-reports of a rewarding "high" and DA increases with intravenous MPH administration in particular (Volkow et al. 1999).

Relatively little research has been done on sex differences in response to MPH treatment. Most research with MPH has been conducted with male rodents due to the greater interactive complexity of female's reproductive cycles (Askenasy et al. 2007, Dafny and Yang 2006). Wooters et al. have shown that adult female rats show significantly greater conditioned locomotor hyperactivity than adult males when given a 3 mg/kg dose of MPH (2006). Because of rising prescriptions in adolescents, and the abuse potential of MPH, findings describing enduring effects from adolescence are especially relevant. Using conditioned place preference (CPP), a behavioral test of reward, chronic exposure to a 2 mg/kg dose of MPH during adolescence showed aversions to cocaine-paired environments in males, but showed preference to cocaine-paired environments in female rats Brenhouse et al. (2009). Furthermore, there are substantial sex differences in the development of DA receptor systems through adolescence. Male rats have

significantly greater overproduction of D1 and D2 receptors in the Striatum and NAc. The increase in male DA receptors during development parallels early motor symptoms of ADHD which may explain the much higher prevalence rate of ADHD in men than women. (Anderson and Teicher 2000). Thus another major element of the studies presented here is to delineate further effects of MPH on sex differences.

Neurotrophins

Psychostimulants such as MPH are known to affect a class of proteins known as neurotrophins (Golden and Russo 2012, Scherer et al. 2010, Messer et al. 2000). Neurotrophins have an important role as neuronal growth factors aiding in maintenance and cell survival as well as synaptic plasticity during development and learning and memory (Bolaños and Nestler 2004). In particular, the neurotrophins Brain-derived neurotrophic factor (BDNF) and Glial cell-line derived neurotrophic factor (GDNF) are important because these have been implicated in a wide variety of brain functions, psychiatric disorders and in the incubation of drug craving (Pickens et al. 2011).

BDNF is expressed throughout the brain. BDNF has notable effects in midbrain dopamine neurons key to motivation and locomotor activity such as the ventral tegmental area in high levels and the NAc and Striatum at lower levels. Thus it has been hypothesized that BDNF is involved in synaptic plasticity changes related to drug addiction and substance abuse (Bolaños and Nestler 2004). Behavioral changes associated with addiction may be a consequence of long-term restructuring of molecular and cellular processes in reward circuits induced by repeated exposure to drugs, essentially a phenomenon of neural plasticity. BDNF activity within the midbrain dopaminergic system plays a critical role in changing plasticity reflected in reward,

sensitization (a factor in the development of drug craving), and drug motivated behavior (Pickens et al. 2011, Horger et al. 1999, Guillin et al. 2001, Nestler 2001).

BDNF functions by activating the tyrosine kinase (Trk) family receptor, TrkB. Activation of Trk receptors leads to several downstream cellular signaling cascades that regulate neuronal excitability, cytoskeletal organization, synaptic connections, and gene expression (Bonni and Greenberg 1997, Bolaños and Nestler 2004, Kaplan and Miller 2000, Pickens et al. 2011). The TrkB receptor is highly expressed throughout dopaminergic neurons in the midbrain, including regions such as the VTA, PFC, and to a lesser extent in the NAc and striatum. Thus, many neurotrophic functions due to BDNF take place in the VTA-NAc circuit (Hyman et al. 1994, Bolaños and Nestler 2004). This VTA-NAc pathway forms the core of the 'motive circuit' and is a major part of the mesolimbic DA system. The primary structures which make up the circuit are the Ventral Tegmental Area (VTA), Nucleus Accumbens (NAc), Ventral Palladium (VP), and Prefrontal Cortex (PFC). Each structure is critical in transforming biological stimuli into adaptive behavioral responses and their functioning is reviewed in greater detail elsewhere (Kalivas and Volkow 2005, Pierce and Kalivas 1997).

Drugs of abuse potently activate the mesolimbic dopamine system and cause long-term neuroadaptation throughout the motive circuit pathways after repeated use (Pickens et al. 2011). Recent evidence has shown that time-dependent BDNF protein levels in the VTA, NAc, and amygdala correlate with time-dependent increases in cocaine seeking during the first 90 days of drug withdrawal (Grimm et al. 2003). Cocaine and amphetamine have also been shown to have significant neural plastic effects on the cell bodies of VTA dopamine neurons. Cocaine increases the number of dendritic branches and spines on neurons in the NAc and the prefrontal cortex. This morphological alteration may persist in drug self-administration in animals for at least one

month after the last drug exposure thus showing evidence of the enduring neuroplastic effects of BDNF (Robinson and Kolb 1997, Robinson et al. 2001).

Furthermore, an increase of BDNF and TrkB signaling is noted in the NAc following both self-administration, and intraperitoneal (ip) administration of cocaine. Conversely, BDNF infusion into the NAc led to increased cocaine self-administration. Thus, dynamic changes of BDNF levels in the NAc promotes addictive behavior, highlighting the profound effects of neuroplasticity within core motivational regions (Graham et al. 2007). Due to the pharmacological similarity of MPH to cocaine these findings are significant and may apply more generally to processes involved with psychostimulant addiction.

The motive circuit's prominent role in processing reward and directing motor action constitute an interface between the limbic and motor systems (Mogenson et al. 1993). Thus, many psychostimulant drugs which activate the mesolimbic dopamine system induce behavioral sensitization. Behavioral sensitization refers to the phenomenon of enduring progressive increases in behavioral response to many drugs of abuse due to chronic exposure to the drug. Evidence shows that repeated exposure to MPH induces behavioral sensitization (Vanderschuren and Kalivas 2000, Yang et al. 2007, Dafny and Yang 2006).

The brain circuitry involved in sensitization is not yet fully understood. However, psychostimulants with rewarding properties have been shown to activate midbrain dopaminergic systems. Blocking D1 and D2 receptors was shown to inhibit sensitization to MPH, indicating that sensitization occurred to rewarding properties of dopamine (Meririnne et al. 2001). Sensitization in other psychostimulants appears to be mediated by DA signaling as well (Vanderschuren and Kalivas 2000). Infusions of BDNF into the VTA dramatically enhance the locomotor sensitization and rewarding effects of acute and chronic cocaine exposure using a CPP

paradigm. In contrast VTA infusions of Nerve-Growth Factor (NGF), another neurotrophin, did not show alterations in morphine or cocaine's actions in the VTA, suggesting that BDNF plays a primary role in sensitization (Horger et al. 1999). Behavioral sensitization may represent early changes in neuronal plasticity in motivation and reward neural circuits underlying long term changes associated with drug addiction. Thus there is substantial overlap between the processes of reward, sensitization, and addiction within mesolimbic circuits, though distinct mechanisms can be identified with various methodologies (Dafny and Yang 2006, Kalivas and Volkow 2005).

Conditioned Place Preference Testing

By using classical conditioning principles, CPP provides a valuable methodology to study drug reward. In CPP the rewarding properties of a drug or non-drug mechanism act as an unconditioned stimulus (US). This treatment is contiguously paired with neutral environmental cues that acquire an association with the natural rewarding properties of the US and act as conditioned stimuli (CS). Subsequent exposure to previously neutral environmental contexts elicits approach behavior if preference was established. Alternatively, motivated withdrawal may occur if conditioned place aversion (CPA) was established (Tzschentke 1998). These response tendencies are evolutionarily pre-programmed as specific reactions to aversive or rewarding stimuli. Increased time spent in the US-paired context indicates preference and thus a rewarding effect, the converse indicates aversion (Bardo and Bevins 2000).

A standard CPP experiment pairs alternative treatments in at least two distinct contexts. A rewarding treatment (drug, food, or other appetitive stimulus) is given while animals are placed into one context. Generally a saline control treatment is given to another animal group in the other context. This pairing can be done a single time or more frequently to mimic chronic stimulus exposure. After an initial conditioning, a post-test round is performed, this time with

animals allowed to freely traverse the three arenas. An increase in time spent in the stimulus-paired context indicates preference and thus, reward (Bardo and Bevins 2000, Tzschentke 1998).

Behavior changes emerge from temporal pairing of environmental context and the stimulus reward. The context environment is a complex multi-modal CS of various sensory cues. Context environments are usually arenas built with variable flooring or coloring on the walls. Texture may also be a feature of the environmental context. Beyond physical cues, researchers must also consider the extent to which novelty as a motivational factor might affect different drug tests (Tzschentke 1998). Because rats prefer novel over familiar environments, this can serve as a confounding factor in CPP testing. Since rats may not have developed familiarization to the context in which drug-pairing occurred, during the drug-free testing day animals may show preference to the drug-paired context due to novelty rather than reward (Bardo and Bevins 2000, Parker 1992). Designing an apparatus with three discrete contexts, a novel, a drug-paired, and a saline-paired compartment can mitigate this bias. During the test day, animals typically show preference to the rewarding drug over the novel environment (Bardo and Bevins 2000). An unbiased procedure was used in the current study.

Because of the contiguous relationship with a conditioned context, CPP is ideal for investigating the temporal profile of reward. Cocaine, for instance, produces CPP if given immediately or five minutes prior to placement in a context, but produces CPA if administered 15 minutes prior, thus revealing thus supporting an opponent-process model of reward in cocaine consistent with self-reports of “high” and generally more negative after effects. Thus CPP's sensitivity to reward allows for delineation of complex CS-US profiles which map to drug pharmacodynamics (Ettenberg et al. 1999).

Critically, CPP measures drug reward, but not reinforcement. Reward refers to the appetitive nature of a stimulus while reinforcement is a contingency that can increase the probability of a behavior (Bardo and Bevins 2000). Furthermore reinforcement paradigms such as self-administration requires multiple drug administrations before reliable behavioral measurements can be obtained which may affect receptor function relating to tolerance or sensitization. A major advantage of CPP is that it appears to be unique in its ability to measure drug reward after single conditioned pairing. Single context US pairing allows for determining drug reward without any induction of tolerance or sensitization. Furthermore physical dependence not required to obtain CPP. (Bardo and Bevins 2000). As a final note on the non-isomorphism of CPP and self-administration, both have been shown to detect reinforcement in a unique set of substances, although there is large overlap (Bardo et al. 1999, Deroche et al. 1999).

Rationale

The goal of these experiments were threefold. The first was to analyze sex differences in CPP with high doses of MPH (5 mg/kg). This dosage is decidedly higher than an established clinical dose so as to represent an abused dosage (Brown et al. 2012). When MPH is abused it is often taken at doses significantly higher than clinical usage. Approximately 75% of MPH abusers take the drug through intranasal administration, thus avoiding first pass metabolism and increasing bioavailability of MPH (Bright 2008, Brown et al. 2012). Previous research has found that MPH reliably produces CPP (Meririnne et al., 2001, Wooters et al. 2011). As a psychostimulant, this is unsurprising. Many other psychostimulant DA-reuptake blocking drugs have been used for CPP as well (Tzschentke 1998). The use of CPP may show sex-differences in the rewarding effects of MPH that align with previous findings on sex-differences in sensitization. In previous work from our lab it was shown that in a clinical dose (1 mg/kg)

resulted in locomotor suppression in both sexes. For higher doses, however (3 mg/kg and 5 mg/kg), female rats showed significantly greater locomotor sensitization (Brown et al. 2012). Others have also found sex differences in MPH sensitization (Chelaru et al. 2012, Wooters et al. 2006). Thus, based off past work it was hypothesized that female rats given a high dose would show more robust CPP than male rats given the same dose (Brown et al. 2012).

A second purpose was to analyze different methodological paradigms of CPP in response to MPH. In the every second day conditioning study, the non-conditioning day may simulate the generally larger time intervals between recreational MPH use (Brown et al. 2012). Additionally, previous work in our lab studying sensitization used an every second day conditioning paradigm for CPP testing. As mentioned above, this paradigm has shown more robust sensitization to a 5 mg/kg dose of MPH in adolescent female rats as compared to male rats. Locomotor tracking showed that sensitization to MPH peaked on the fifth day of treatment (Brown et al. 2012).

This is interesting because past research has shown that heightened sensitization typically indicates more robust dopamine responses in brain areas associated with drug reward (Vanderschuren and Kalivas 2000, Kalivas and Duffy 1993). Brain plasticity changes reflecting reward and the incubation of drug craving may arise during drug withdrawal. Previous work has shown increased sensitization in response to MPH, related to underlying mechanisms involving BDNF and reward (Brown et al. 2012) Thus, it was hypothesized that brain plasticity changes in reward circuits would occur during the non-testing days, thus showing more robust CPP in females as compared to males which reflect changes observed in sensitization (Cummins et al. 2013).

A final purpose was to examine the effects of MPH on DAT levels in male and female adolescent rats. Blockage of the DAT is the primary mechanism of action of MPH, however

research has shown a decrease in the actual density of the DAT following MPH treatment in both adults and adolescents (Izenwasser et al. 1999, Moll et al. 2001). Research has shown a dose-dependent decrease in striatal DAT to MPH. However, DAT levels in the NAc were not tested (Nikolaus et al. 2007). The NAc is of primary importance for drug reward and plays a central role in the function of the motive circuit (Pierce and Kalivas 1997). Lower levels of the DAT will have a direct impact on dopaminergic activity in the synapse. Doses more relevant to abused MPH may show greater changes in DAT levels. Furthermore, there is no data on sex differences in DAT density due to MPH exposure. Past research has shown sex differences in conditioning due to MPH as well as sex differences in behavioral response and dopaminergic function to psychostimulants (Wooters et al. 2006, Becker 1999). Thus we hypothesized that females would show a decrease in the DAT levels in the Dorsal Striatum and the NAc compared to male rats.

Methods

Two experiments were conducted to determine the effects of MPH on CPP. In the first experiment, saline control animals and animals given a clinically relevant 1 mg/kg dose were compared to 5 mg/kg, a dose more relevant to abuse. DAT was measured from animals in the first experiment. In the second experiment, all animals were either given a 5 mg/kg dose or saline as a control. The CPP paradigm in the second experiment was switched to an every-other conditioning day paradigm.

In each group 8 Sprague-Dawley rats were used. The gender of the rodents was split evenly in all groups. Rats were tested during adolescence, defined as development between postnatal days (P)28 to P60 for rats (Smith 2003). Conditioning in the first experiment took place every day from P44-48. The second experiment was conducted every second day during days P33-41. This was done so that a more complete range of adolescent development could be tested.

For CPP testing in these studies a three-chambered apparatus was used with identical dimensions in three visually distinct environmental contexts. Locomotor arenas were rectangular wooden boxes with square compartments measuring 30 cm on a side. An additional compartment was used to provide novelty in addition to the drug-paired compartment and saline-paired compartment to eliminate the confounding effect of novelty-seeking in rats (Bardo and Bevins 2000). Removable dividers separated each context. Tactile surface was also distinct in each context.

For the first experiment animals were given an initial preference test on P43.This was to determine if there was an initial context which was preferred by the animal. An ip Saline injection was given and the animals were placed into the apparatus without context dividers and allowed to freely explore for a 10 minute period. No initial context preference was demonstrated by any group (Cummins et al. 2013).

Beginning the day after initial preference testing, dividers were replaced into the apparatus and conditioning tests were conducted. Each animal was assigned randomly to the horizontal or vertical environmental context. For animals given MPH, the context temporally paired with MPH was the paired context, the saline-paired context was the unpaired context. Control animals were given saline in both contexts. On each conditioning day a morning session was done where all animals were given saline and placed into their initial assigned context for a 10 min trial. In the afternoon session animals in the MPH group were administered either 1 mg/kg or 5 mg/kg MPH and 10 min later placed into their assigned context for a 10 min trial. Assignment of paired contexts were balanced across animals to prevent bias (Cummins et al. 2013).

After conditioning trials were complete, a post-conditioning preference test was conducted on P49. This test was identical to the initial preference test, with dividers removed. All animals were administered ip of saline to control for stress of injection. Animals were then able to freely explore the apparatus. Animal behaviors were recorded as horizontal activity. The computer program Anymaze (from Stoelting Co, Woodale, IL) superimposed a grid on a digital video feed of the animals. Each time rats crossed a line it was counted as a locomotor activity count. During the post-conditioning preference test, the time spent in the paired context was divided by the time spent in both the paired and unpaired context as measured by Anymaze. This determined the preference ratio of the animals (Cummins et al. 2013).

To measure the effects of chronic MPH on the DAT in adolescence, one day after the post-conditioning test was conducted the animals in the consecutive-day paradigm were sacrificed. The dorsal striatum and NAc were dissected out of the brain and brain and tissue was analyzed. Analysis of DAT was conducted using a Western Blot assay. Measurement of the DAT was only performed on animals given 5 mg/kg because these showed CPP (Cummins et al. 2013).

The second experiment was conducted with slight methodological differences. Again, 8 Sprague-Dawley rats were used in each experimental group in even gender proportions. Conditioning days in this experiment were conditioned using an every second day paradigm. Conditioning for this experiment was identical to the first experiment, with a few exceptions. A clinical dose of 1 mg/kg was not used. Instead only saline control animals and 5 mg/kg MPH animals to directly compare the every second day paradigm with the every day paradigm in the context of an abused dose. Our lab had not shown sensitization to a clinical dose previously, so this dosage was not tested in the second experiment. The testing days occurred from P33-43,

with conditioning on every second day in order to test a wider developmental period. Each conditioning day had two sessions conducted identically to the first experiment (Cummins et al. 2013).

Results

In the first experiment, the preference ratios of rats given 1 mg/kg were not statistically different than those of rats given saline (Fig. 1). However, rats given the high 5 mg/kg dose showed significantly higher preference ratios, thus showing dose-dependent CPP to the drug-paired contexts. Both male and female rats showed significantly greater CPP at this dose, but there was not a statistically significant sex difference between the two groups. Thus the hypothesis that CPP would mirror sex differences in sensitization based on previous findings was not shown to be true (Cummins et al. 2013). A two-way ANOVA (sex, drug) of the preference ratio revealed a significant main effect of adolescent drug treatment $F(2,47)= 6.15$, $p <.005$, but there was no significant main effect of sex nor an interaction of sex on adolescent drug treatment (Cummins et al. 2013).

For DAT density testing, a 5 mg/kg dose of MPH administered for five consecutive days showed significant effects on both the striatum NAc (Fig. 2). The Western Blot assay showed a significant decrease in the DAT in both the NAc and dorsal striatum. In females, DAT in the dorsal striatum was shown to decrease by 46.4%, while males showed a 44.3% decrease. In the NAc there was a 31.5% decrease in DAT for females and a 48.2% decrease for males. No prior studies have reported on the effects of MPH on DAT density in adolescence. There were no significant effects on DAT levels between genders (Cummins et al. 2013). A two-way ANOVA for the dorsal striatum resulted in a significant main effect of adolescent drug treatment $F(1,34) = 6.53$, $p < .016$, but there were no significant main effects of sex or a significant interaction of sex

on adolescent drug treatment. For the NAc, a two-way ANOVA revealed a significant main effect of adolescent drug treatment $F(1,34) = 6.16$, $p < .019$, but again, no significant main effects of sex or an interaction of sex on adolescent drug treatment were found.

Interestingly the methodological change from a consecutive day conditioning paradigm of the first experiment to an every second day conditioning paradigm in the second experiment did not reveal significant sex differences in place preference (Fig. 3). A significant preference ratio was still shown in response to a 5 mg/kg dose. This difference was notable in that it was slightly lower, though not significantly so from the consecutive day 5 mg/kg group (Cummins et al. 2013). A two-way ANOVA revealed a significant main effect of adolescent drug treatment $F(1,31)=10.59$, $p<.003$. There was no significant main effects of sex nor a significant effect of sex on adolescent drug treatment. While MPH produced a significant increase in preference ratio in both male and females, no significant sex differences were found (Cummins et al. 2013).

Discussion

These experiments reveal several important findings related to the rewarding properties of MPH. As noted before, when taken recreationally, MPH may not be used consecutively. Additionally the greater dosage and ip route of administration is more relevant to an abused dose (Brown et al. 2012). Our results show that a similar magnitude of place preference can be established with non-consecutive MPH exposure in rats. The dopamine deficit mentioned before is a generally accepted model of ADHD (Tripp and Wickens 2009). However, an animal model of ADHD was not used here. Thus these findings may be of more relevance to recreational use of MPH.

The results of the consecutive day CPP experiment showed that place preference to a 5 mg/kg dose of MPH is not significantly modulated by gender. This is in contrast to our

hypothesis because previous findings in our lab and others, have revealed sex differences to MPH sensitization at 5 mg/kg (Brown et al. 2012). Chelaru et al. (2012) also found sensitization to be enhanced in female rats as compared to male rats at a 2.5 mg/kg dose. Thus it is clear that there are sex differences in behavior in response to MPH. These findings highlight the differences between CPP and sensitization, despite the reliance of both on mesolimbic dopaminergic activity. Sensitization represents the arousing effects of drugs of reward, while CPP denotes reward in relation to a particular context. Further research will reveal the complex interaction of reward and gender.

Western blot analysis of DAT levels showed reduction of DAT densities in the dorsal striatum and NAC. This may result in increased availability of synaptic dopamine in these areas. This would lead to an enhanced rewarding effect. Thus recreational use of MPH in adolescents may experience greater reward and thus be in greater danger for addiction than in clinically relevant usage of MPH. A review of the incubation of drug craving can be found in Pickens et al. (2011). Reduction of the DAT in reward areas has the additional consequence of affecting the long-term development of the brain's reward system. Other studies have shown that prenatal exposure to MPH results in an increased response to rewarding stimuli into adulthood (Crawford et al. 2007). The long-term effect of reduced DAT during adolescence has yet to be fully studied, but may play a role in in this effect.

The results of the every second day CPP paradigm indicate that conditioned place preference in response to a 5 mg/kg was not significantly affected by a modified dosing regimen. The small age difference between the two rat groups may appear irrelevant, but the adolescent model represents a broad period of rapid dopaminergic development (Andersen & Teicher, 2000). Slightly different behavioral changes were noted between the two CPP paradigms,

however the magnitude of reward was not significantly affected across the wider developmental period tested (P33-49 for both groups combined) or the spaced dosing regimen.

In conclusion, the research presented here reviews experiments conducted in our lab on the effects of MPH on CPP. A high dose, representing an abused dose of MPH recreationally, produced CPP in both male and female adolescent rats. There were no sex differences in the preference ratio for animals treated with MPH. Furthermore, CPP occurred at the same magnitude regardless of MPH administration by a consecutive day or every second day exposure paradigm. This high dose of MPH also produced significant decreases in the DAT in both the dorsal striatum and NAc, key reward centers of the brain. Thus an increase in overall dopaminergic activity can occur, increasing the potential for addiction to MPH. Changes in the DAT system in adolescents may also lead to altered response to behavior later in life. Despite the lack of significant sex-differences to CPP in this study, there are clear sex differences in behavior to MPH. Further research will help clarify the complex relationship between gender, age, and addiction.

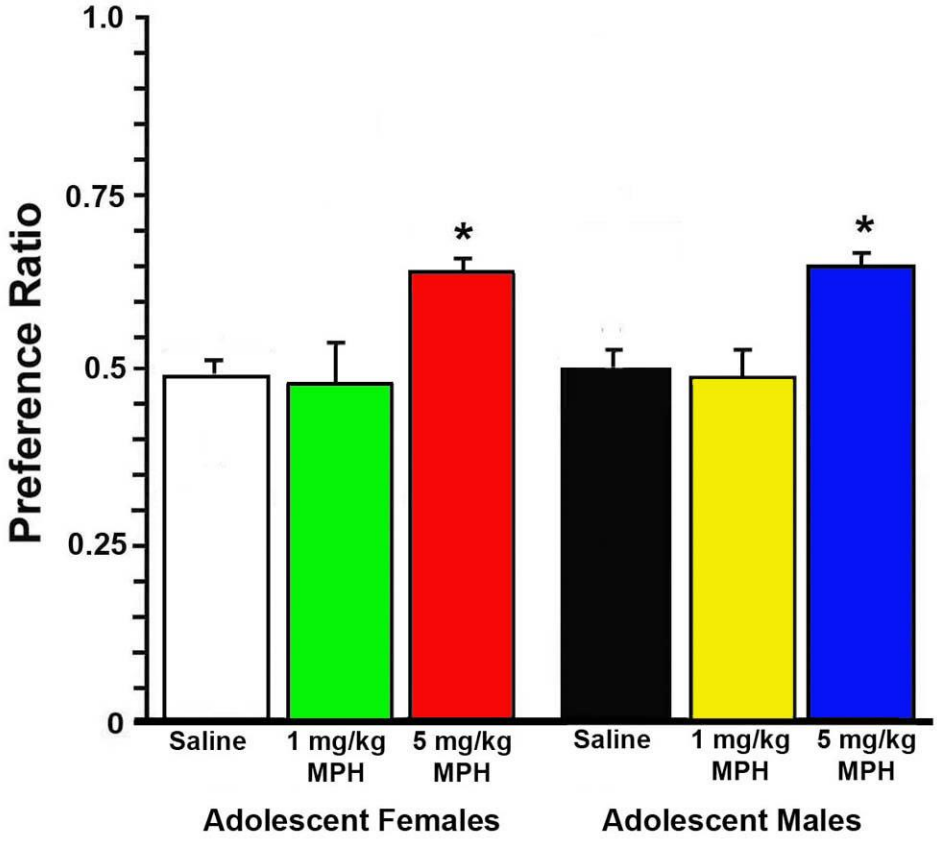

Fig. 1. Preference ratio is presented as a function of group for the post-conditioning preference test in the consecutive day paradigm. Asterisk (*) indicates that the group mean was significantly higher than the 1 mg/kg MPH group and the saline control group.

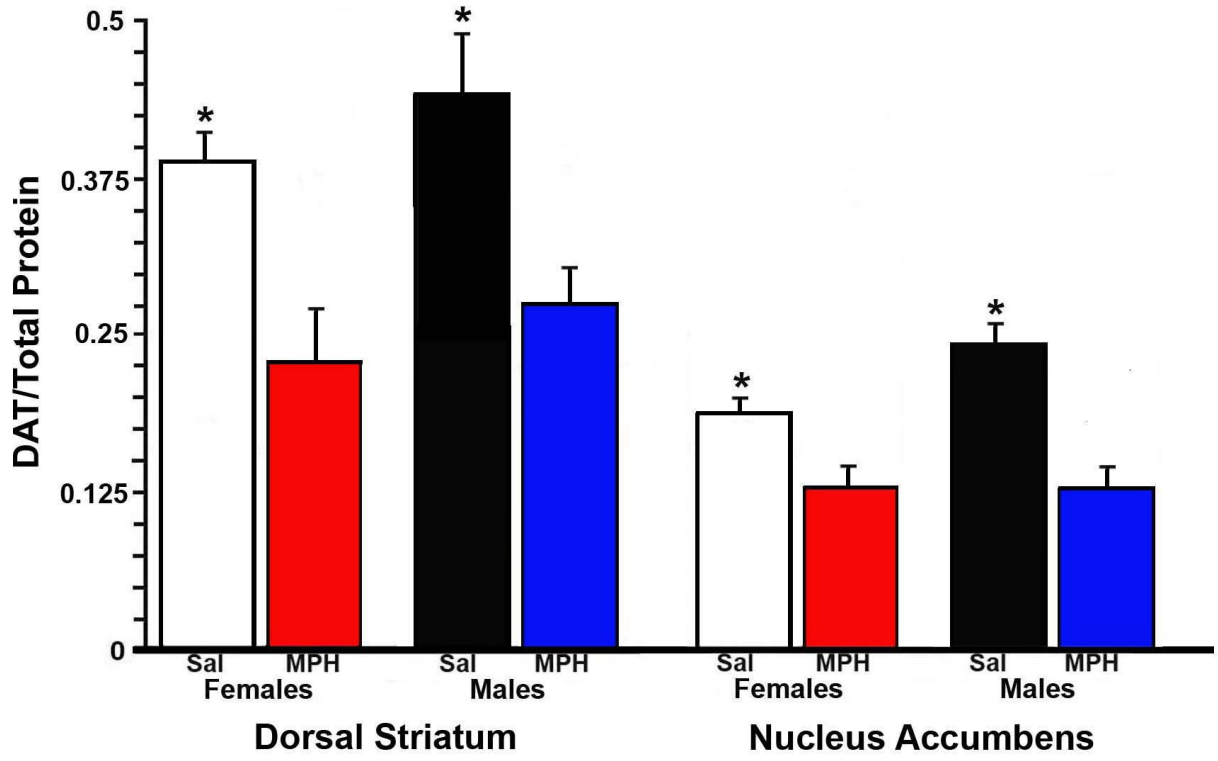

Fig. 2. The DAT is presented as a ratio to total protein for both brain dorsal striatum and NAc. Asterisk (*) indicates that control group mean was significantly higher than drug treatment group.

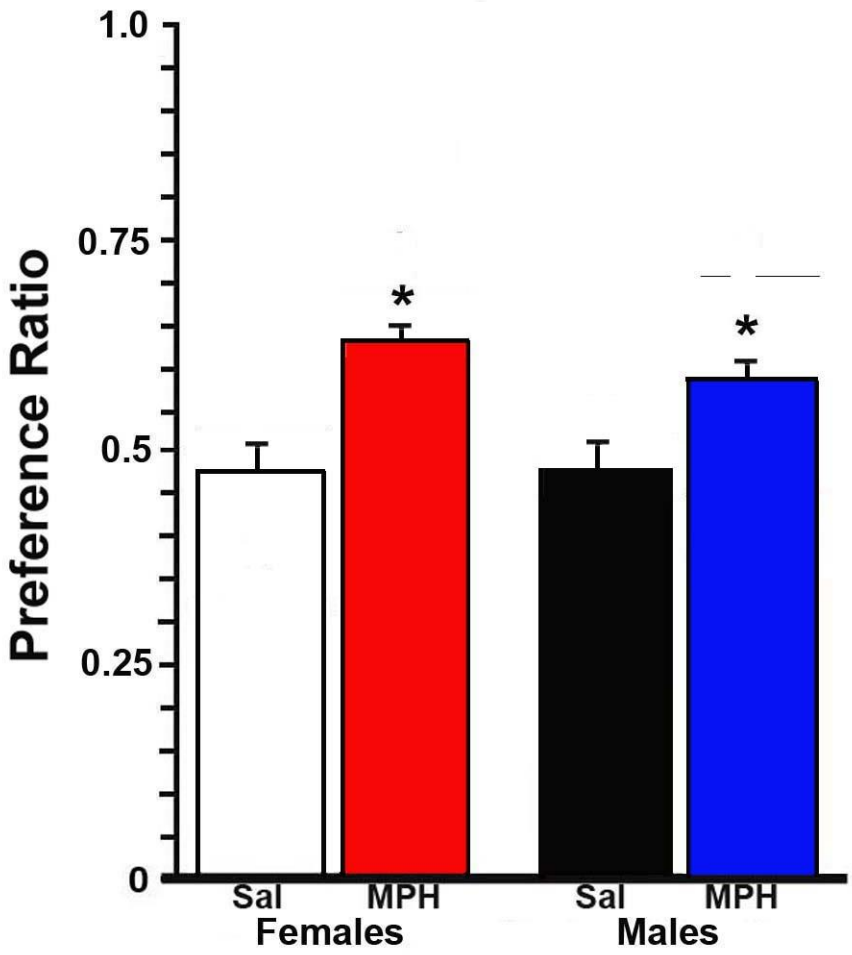

Fig. 3. Preference ratio is presented as a function of group for the post-conditioning preference test in the every second day paradigm. Asterisk (*) indicates group mean was significantly greater than saline control group.

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