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The Effects of Clozapine on Methylphenidate-Induced Conditioned Place Preference

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Methylphenidate (MPH) is commonly prescribed for attention deficit/hyperactivity disorder (ADHD). It is similar to cocaine in that it inhibits the dopamine transporter to elevate extracellular dopamine levels and has reinforcing effects. As ADHD diagnoses have increased, MPH abuse has increased as well. There is evidence that DA antagonists such as Clozapine may be effective in mitigating cocaine abuse. Therefore, it is hypothesized that Clozapine will inhibit the rewarding effects of MPH in a conditioned place preference (CPP) paradigm. The data showed that both MPH and Clozapine treated groups had a significant aversion to the drug-paired compartment. Because MPH produced a conditioned aversion, the effect of Clozapine on the rewarding effects of MPH remains to be elucidated. Future studies using lower doses of MPH, as well as those analyzing dopamine levels in the striatum and prefrontal cortex would provide evidence of the effect of Clozapine on mesolimbic dopamine systems.

Keywords*:* methylphenidate, clozapine, conditioned place preference

Introduction

There is not one definition that concisely defines addictive behavior. However, when pertaining to drug addiction, Seymour and Wagner (2008) defined addiction as a behavioral syndrome, characterized by compulsive drug seeking with repeated relapses into drug use. Addictive behavior following repeated exposure to drugs is believed to be mediated by sensitization enhanced behavioral response following repeated administration of drugs of abuse (Seymour & Wagner, 2008). Sensitization to drugs of abuse, such as methylphenidate, (MPH) which is a psychostimulant is thought to be one aspect of the cycle of addictive behavior. Sensitization affects behavior through learned associations with the drug experience such as where the drug was taken, time of day, or certain noises or music. It has been found that sensitization and neurochemical effects also underlie components of drug cravings (Beyer & Steketee, 1999) which ultimately leads to addiction. Disruption of monoamine neurotransmitters, specifically dopamine (DA), mediates addictive behavior (Rothman, Blough, & Baumann, 2006). More specifically, the mesolimbic DA pathway is altered by repeated exposure to drugs of abuse. MPH initially increases extracellular DA levels by blocking reuptake. However, after continued MPH abuse, the DA levels start to deplete. As such, reduced activity in the mesolimbic system is thought to mediate the intense drug cravings characteristic of psychostimulant abuse (Dackis & O'Brien, 2001). Due to the similarities between MPH and cocaine, they may have similar mechanisms that induce drug cravings and influence people with drug addictions to continue to abuse drugs.

A major model for understanding cocaine addiction or psychostimulant addiction in general is the dopamine depletion hypothesis (Dackis & Gold, 1985). The dopamine depletion hypothesis can be studied using the conditioned place preference (CPP) model because you can easily observe the interaction between the rewarding effects of cocaine and conditioned cues that strengthen addiction. Cocaine initially increases DA levels, producing a rewarding euphoric feeling that becomes associated with an environmental cue that the user saw, heard, or felt during drug use, through principles of classical conditioning. When a strong rewarding effect, such as euphoria, is connected to an environmental cue it strengthens that association. When the drug wears off and the user begins to experience a craving for the drug, any of the conditioned environmental cues that were associated with the rewarding effects will prepare the body for the cocaine. The environmental cue is now conditioned stimuli for the unconditioned response of euphoria. This can be related to MPH because of the many similarities between the two drugs. MPH produces a comparable increase in synaptic DA levels which in turn causes rewarding effects (Rush & Baker, 2001).

The dopamine depletion hypothesis is now a well established model that maintains decreases in synaptic DA levels are a main mechanism for drug addiction (Dackis & Gold, 1985), however, it is not the only model that explains drug addiction. Different drugs affect DA in different ways; some drugs act by augmenting the presynaptic release of DA, whereas some drugs inhibits reuptake (Gill et al., 1991). MPH, in particular, blocks reuptake and increases synaptic DA levels and DA neurotransmission (Kollins, MacDonald, & Rush, 2001). Increased dopamine levels disrupt the regulatory mechanisms for DA production and release. The D2 autoreceptor acts as a feedback mechanism for maintaining neurotransmitter release by regulating the firing rate of DA synthesis and release (Parish et al., 2005). MPH increases DA levels which activates D2 autoreceptors to return DA levels to normal (Federici, Geracitano, Bernardi, & Mercuri, 2005). If MPH abuse continues; it causes the D2 autoreceptors to stay activated, which leads to dopamine depletion (Federici et al., 2005). The large amount of DA that is lost due to overactivation of D2 autoreceptors causes a much higher demand for DA synthesis which is inefficient in compensating for the loss. The inability to compensate for the loss of DA production decreases DA levels in the synapse. As synaptic DA levels

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are depleted, there is a significant increase in feelings of disphoria that increase the craving for cocaine which mediates drug-seeking behavior.

The dramatic neurochemical changes involving dopamine are a major neurobiological correlate of addiction. The positive and negative reinforcing properties of MPH contribute to its abusive properties (Seeman & Madres, 2002). The positive reinforcing properties of MPH are believed to be attributed to acute stimulation of DA neurotransmission in the mesolimbic dopamine pathway in the ventral tegmentum region (Rosa-Neto et al., 2005). These reinforcing effects include increased energy, enhanced alertness, enhanced sensory experience, and rewarding euphoric feelings (Wee & Woolverton, 2004). The anhedonic effects of MPH can include decrease neural activity, anxiety, loss of concentration, disrupted movements, impaired learning, and impaired skill acquistion (Easton, Marshall, Marsden, & Fone, 2009). Additionally, as the drug effects dissipate, there is a prolonged feeling of dysphoria that is often mediated by depleted dopamine levels and is often profound enough to promote further drug seeking and use (Easton et al., 2009).

Since people who abuse MPH have decreased DA levels due to it's agonistic effects and Clozapine is a DA antagonist, concurrent use of Clozapine and MPH could decrease euphoric effects of MPH which could also decrease its abuse potential (Wyatt, Karoum, & Masserano, 1998). Studies have been done to determine exactly how Clozapine affects DA levels. Thus far, it has been found that Clozapine blocks post-synaptic D2 receptors and increases activity of feed-back loops (Wyatt et al., 1998). Since Clozapine attenuates D2 autoreceptor activiation, it in turn, increases DA synthesis and release while returning DA levels back to normal (Parish et al., 2005). Therefore, the current study is testing the hypothesis that Clozapine will inhibit MPH-conditioned place preference, a well established behavioral model of addiction.

There are a variety of approaches to studying the sociological, behavioral, and pharmacological aspects of addictive behavior. Studying the behavioral and pharmacological aspects of addiction can lead to a better understanding of how these behaviors emerge from conditioned environmental cues. CPP is a paradigm that can be easily used to study those relationships. The research presented evaluates how pharmacological interventions can block or attenuate conditioned place preference for drugs of abuse or psychostimulants. This research is focusing specifically on MPH abuse which may lead to addiction. MPH is one of the most commonly prescribed psychoactive drugs in the United States, about 11 million prescriptions per year (Kollins et al., 2001). Due to the ease of obtaining MPH legally, it has become widespread among adolescents and college students. One in five children that are prescribed MPH to treat their attention deficit/hyperactivity disorder (ADHD) are approached to sell, give away, or trade their medication (Kollins et al., 2001). The increase in misuse and abuse of MPH has already lead it to obtain street names such as "Vitamin R", "Skippy", and "the smart drug" (Kollins et al., 2001). There has been an increase in theft of MPH in schools and pharmacies and scams involving MPH. It is not uncommon for people to obtain multiple prescriptions from different doctors through scams then sell or trade it illegally (Kollins et al., 2001). Its structural and pharmacological similarity to drugs such as cocaine gives more reason to believe MPH has a significant abuse potential (Kollins et al., 2001).

Place conditioning is commonly used to study the rewarding and incentive motivational effects of drugs (Zavala, Weber, Rice, Alleweireldt, & Neisewander, 2003). Place conditioning which is based on classical conditioning involves repeatedly pairing an unconditioned incentive (e.g., MPH) with a distinct environment (e.g., nonpreferred or drug-paired compartment) then allowing free access to both compartments in absence of the incentive. If an animal spends more then 65% of its time in the compartment that was paired with the incentive, a conditioned place preference has been established toward that compartment due to the association (Seymour & Wagner, 2008). The minimum standard, 65%, is used because less than that would suggest the results were due to chance (Seymour & Wagner, 2008). This study used MPH as the unconditioned incentive. If Clozapine inhibits the MPH-induced behavioral and neurochemical effects, there will be no preference toward the compartment that was paired with the MPH.

CPP is a design that can effectively test the hypothesis that Clozapine will attenuate MPH-induced place preference. Drugs that decrease MPH's depletion of DA will attenuate MPH-induced conditioned place preference. More specifically, Clozapine, a DA antagonist, is being studied to determine if it inhibits MPH-induced conditioned place preference. There are four specific predictions being studied in this research. Rats injected with MPH10 will show a conditioned preference to the drug-paired compartment; Clozapine, 25 mg/kg, plus MPH10 will produce an aversion to the drug-paired compartment; MPH5 will show a stronger conditioned preference to the drug-paired compartment than MPH10; and saline-injected rats will not show a conditioned preference to either the drug-paired or non-drug paired compartment.

Methods

Subjects

Long Evans Hooded female rats of about four to six months old were used in this study. The 21 rats were housed in separate cages and had free access to food and water. The procedures used are in accordance with the APA Ethical Guidelines for the Care and Use of Laboratory Animals.

Apparatus

This research used a conditioned place preference (CPP) apparatus built by the researcher using measurements taken from recent studies (Allen, Everett, Nelson, Gulley, & Zahniser, 2007; Busse, Lawrence, & Riley, 2004; Kosten & Nestler, 1994; Seymour & Wagner, 2008; Zavala et al., 2003). It contained two large compartments connected by one small compartment. The dimensions for the two large compartments were 30cm x

20cm. One compartment had black walls with a wood floor and bedding. The second compartment had white walls. The dimensions for the middle compartment were 20 x 20 cm. This compartment had gray walls with a wood floor. There were clear plastic doors separating the two compartments. These doors could be removed when needed. There was also a clear plastic roof in order to easily observe the rats. The apparatus may be seen in Figure 1.

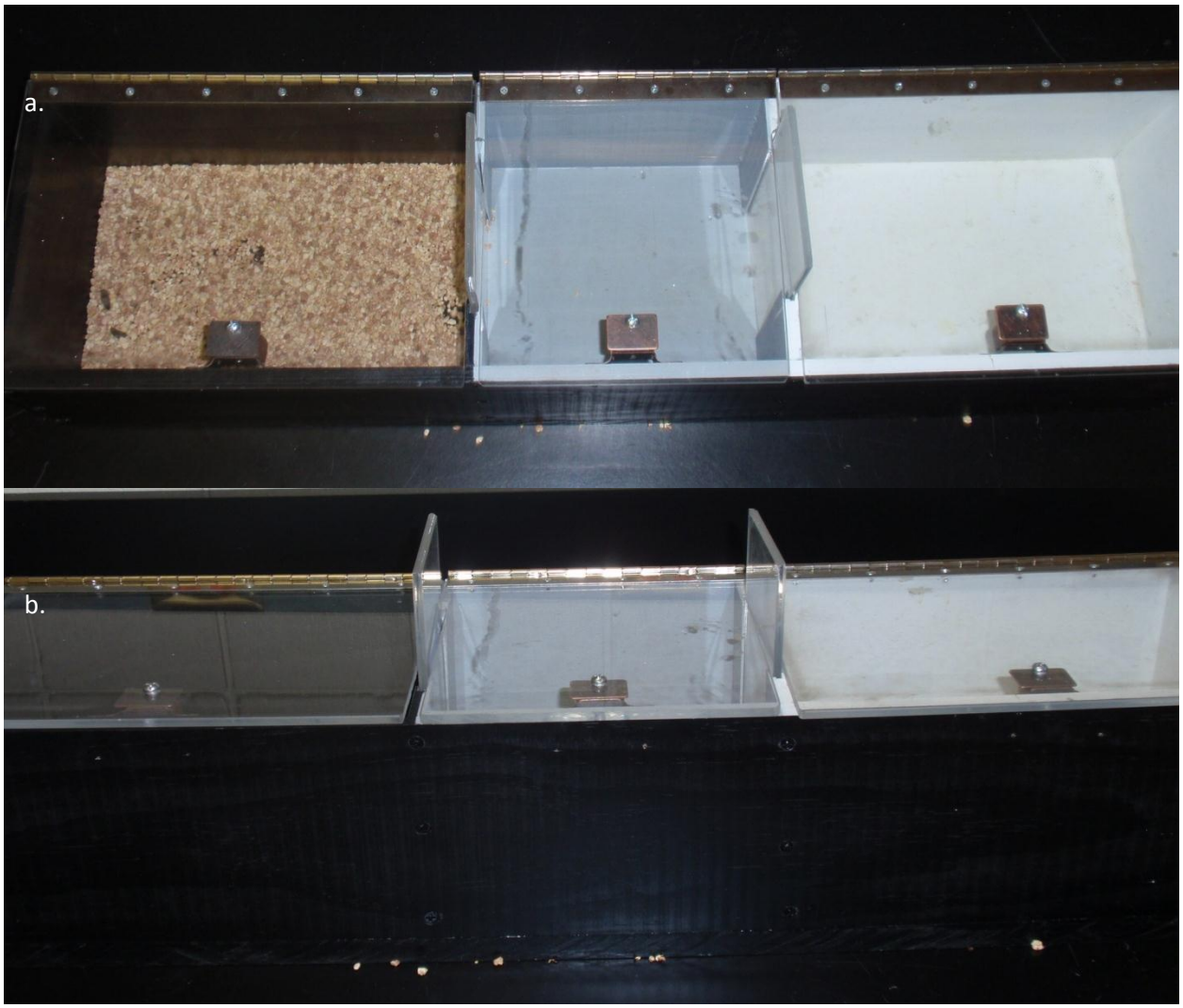

Figure 1: a. Side view of the CPP apparatus. b. Top view of the CPP apparatus.

Procedure

The research made use of a between subjects design with 21 rats. Each rat was tested for one week and received one of the four treatments. The first group of seven rats that received saline later on received a 5mg/kg dose of MPH as well. MPH5 was later tested to determine if there was a difference between MPH injected at 10 mg/kg and at 5 mg/kg.

Adaptation

On day one of week one, the rats were assessed in the CPP apparatus for their baseline preference. They were placed in the middle compartment with the clear plastic doors open to allow free access to both compartments. The animal's behavior was recorded for 20 minutes using a video recorder and the amount of time spent in each compartment was then determined. When their head crossed the door, time began and ended. The compartment in which they spent more than 65% of their time was their preferred side. In the case of the percentage being less than 65%, the compartment in which they spent a greater percentage of time was used as their baseline preference. This adaptation procedure was repeated for every rat.

CPP Training

The rats were trained in their nonpreferred side to avoid bias. Each week the rats were given injections of one of the four drug treatments. Drugs were injected intraperitoneally (ip) and included saline (Sal), methylphenidate (MPH10; 10 mg/kg and MPH5; 5 mg/kg), or Clozapine plus methylphenidate (ClozMPH10; 25 mg/kg and 10 mg/kg, respectively) (Broderick, Hope, Okonji, Rahni, & Zhou, 2004; Farren et al., 2000). The injections were given in their nonpreferred side; the opposite compartment of their baseline preference. This was followed by confinement to that compartment for 20 minutes. They were trained once each day for four days.

CPP Testing

The day after CPP training was completed on day six, each rat was put in the middle compartment with the clear plastic doors open to allow free access to both compartments. The animal's behavior was recorded for 20 minutes using a video recorder and the amount of time spent in each compartment was then determined. The side the rat spends more than 65% of their time was their new preferred side, which is their conditioned preference.

Data Analysis

This study used a one-way ANOVA to analyze data between groups, comparing the percentage of time spent in the non-drug paired compartment and the drug-paired compartment. It also used a paired samples t-test to analyze data within groups comparing the percentage of time spent in the non-drug paired compartment with the drug-paired compartment post-treatment. A second paired samples t-test was used to analyze data within groups comparing the preference toward the drug-paired compartment pre-treatment with post-treatment.

Results

This study tested 21 rats to determine the effect of Clozapine on MPH-induced conditioned place preference. Four hypothesis were tested: MPH10-injected rats would show a conditioned preference to the drug-paired compartment; Clozapine plus MPH10-injected rats would show an aversiont to the drug-paired compartment; MPH5-injected rats would show a stronger conditioned preference to the drug-paired compartment than MPH10; and saline-injected rats would not show a conditioned preference to either the drug-paired or non-drug paired compartment.

A one-way ANOVA was used to analyze data between groups, comparing the percentage of time spent in the non-drug paired compartment and the drug-paired compartment post-treatment. There was a significant difference in preference to the non-drug paired compartment, F (3, 22) = 6.331, p = 0.003, η^2 = 0.460. The Tukey HSD showed that Clozapine plus MPH10-injected rats spent significantly more time in the non-drug paired compartment than saline-injected rats, (M = 82.0, SD = 10.173). Clozapine plus MPH10-injected rats also spent significantly more time in the non-drug paired compartment than MPH5-injected rats, (M = 82.0, SD = 10.173). These results can be seen on Figure 2. Saline-injected rats showed no sigificance in both the drug-paired and non-drug paired compartment, $p > 0.050$.

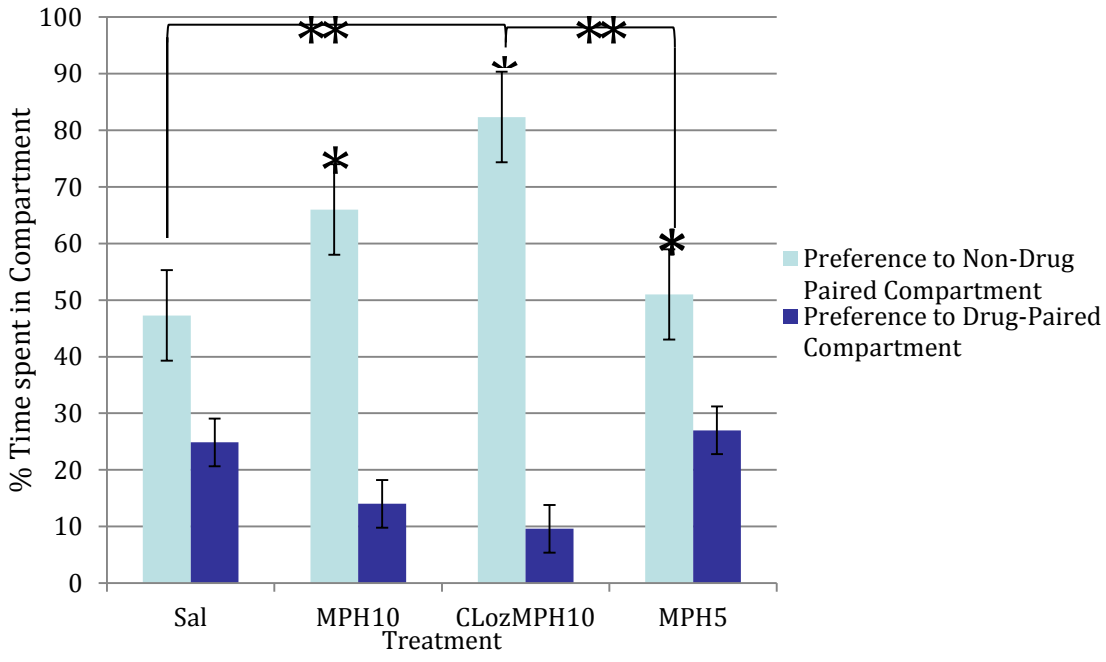

Figure 2:. Preference in non-drug paired compartment compared with preference in drug-paired compartment post-treatment. Error bars represent the standard error. One asterisk represents significance within groups ($p < 0.050$). Two asterisks represent significance between groups ($p < 0.050$).

A paired samples t-test was used to analyze data within groups comparing the percentage of time spent in the non-drug paired compartment with the drug-paired compartment post-treatment. The MPH10, Clozapine plus MPH10, and MPH5 groups all spent significantly more time in the non-drug paired compartment than in the drug-paired compartment post-treatment, t (6) = 4.600, p = 0.004, t (4) = 10.500, p = 0.000, and t (6) = 2.700, p = 0.037; respectively. These results can be seen on Figure 2.

A paired samples t-test was also used to analyze data within groups comparing the percentage of time spent in the drug-paired compartment pre- and post-treatment. The MPH5 group spent significantly more time in the drug-paired compartment post-treatment, t (6) = -2.500, p = 0.042 (see Figure 3). As seen in Figure 3, the MPH10 and Clozapine plus MPH10 groups spent less time in the drug-paired compartment post-treatment, however; this result was nonsignificant.

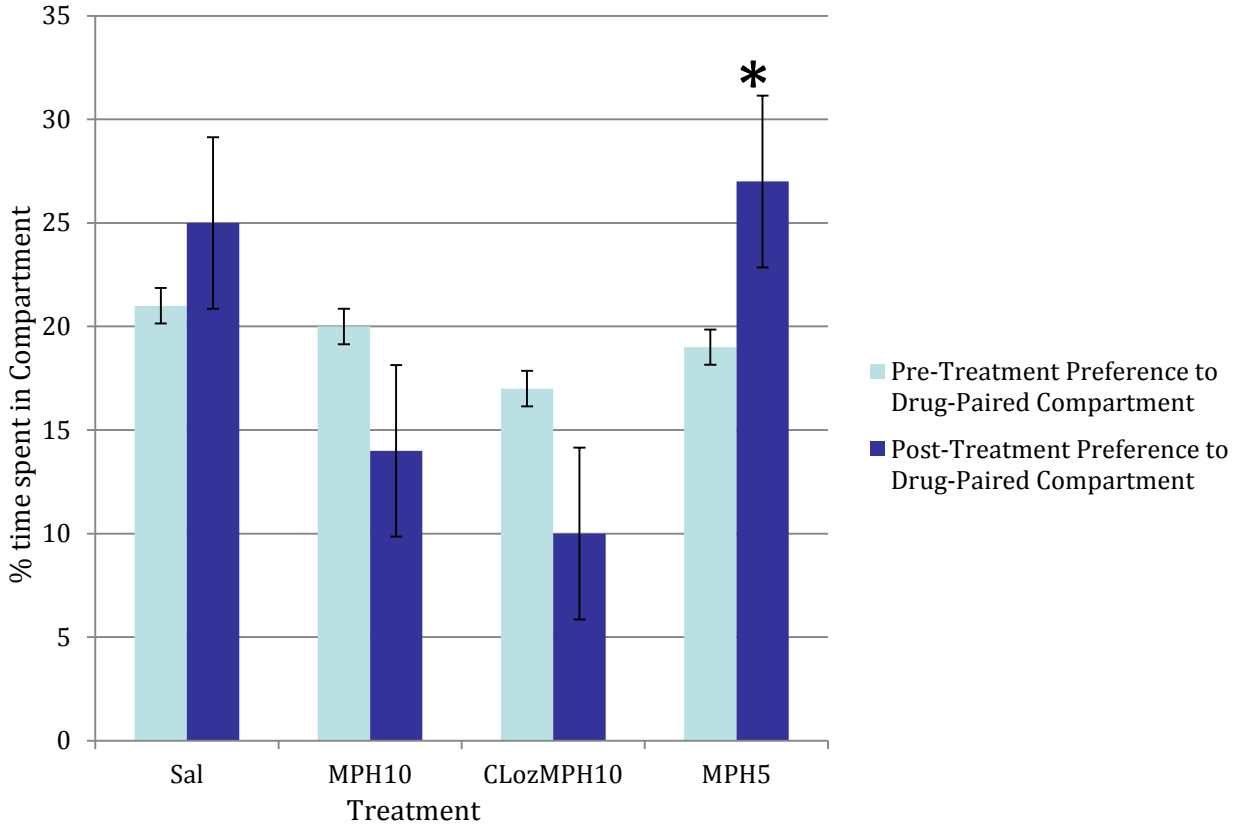

Figure 3: Percentage of time spent in the drug-paired compartment pre- and post-treatment. Error bars represent the standard error. One asterisk represents significance within groups ($p < 0.050$)

DA pathway. However, it was determined in this study that much lower dosages of MPH than cocaine are needed to induce a conditioned preference. This implies that the abuse potential for MPH is actually higher than cocaine because it doesn't take as much of the drug to produce a conditioned preference. This interpretation conflicts with what other studies have found. Broderick et al. (2004) and Farren et al. (2000), for example, found that cocaine given at 10 mg/kg was the optimal dose to produce a conditioned place preference, therefore, a 10 mg/kg dose of cocaine has become a research standard. Zhu, Spencer, Liu-Chen, Biederman, and Bhide (2011) determined that higher doses of MPH produced a significant conditioned preference to the drug-paired compartment, but lower doses did not. They compared a 7.5 mg/kg and a .75 mg/kg dose of MPH with a 10 mg/kg dose of cocaine. They also found there was no significant difference between the high dose of MPH and cocaine in the strength of the conditioned preference to the drug-paired compartment. Work done by Zhu et al. (2011) suggests that a high dose of MPH was essentially as rewarding as cocaine and the abuse potential was similar. Wooters, Walton, and Bardo (2011) compared a 3 mg/kg and 10 mg/kg dose of MPH using a CPP apparatus. They also found that the higher dose was more effective at producing a significant conditioned preference to the drug-paired compartment than was the lower dose.

In addition to the high dose, anxiolytic effects produced by overstimulation of the DA and sympathetic nervous systems could have contributed to the aversion caused by MPH10 as well. This could cause problems in patients who are prescribed MPH for ADHD. If patients with ADHD do not receive the correct dose, MPH could be overstimulating, causing aversive side effects. Such side effects include: difficulty in speech and eating, suicidal thoughts, increased aggressiveness and agitation, hallucinations, psychosis, lethargy, seizures, tachycardia, dysrhythmias, and hypertension (Rodriguez et al., 2010).

These aversive side effects could lead the patient to stop taking medication.

It is unclear why Clozapine plus MPH10 produced an aversion to the drug-paired compartment; however, there are some possible reasons. The aversion could have been due to the hypothesized inhibiting effects of Clozapine on MPH. MPH is a DA agonist whereas Clozapine is a DA antagonist; the drugs are working in opposition to each other on the same DA pathway. Therefore, it was hypothesized the Clozapine would inhibit the MPH-induced conditioned place preference. The aversion produced by Clozapine plus MPH10 is consistent with a study done by Kleven, Prinssen, and Koek (1996). In their study, Clozapine was shown to inhibit MPH-induced behaviors and movements. Clozapine has many aversive side effects that could have also caused the aversion to the drug-paired compartment. These side effects include: seizures, sedation, weight-gain, nasal congestion, excessive salivation, orthostatic hypotension, tachycardia, and hyperglycemia (Wu et al., 2000; Farren et al., 2000). Lastly, Clozapine was given with MPH10. MPH10's high dose or overstimulating effects could have over powered Clozapine's effects on the DA pathway causing the aversion.

MPH5 showed a significant increase in preference toward the drug-paired compartment from pre- to post-treatment. Despite the increase in preference, animals injected with MPH5 still spent more time in the non-drug paired compartment producing an aversion. This was unexpected because other studies have demonstrated that although not significant, lower doses of MPH did produce a conditioned preference (Wooters et al., 2011; Zhu et al., 2011). The aversion caused by MPH5 could be attributed to the amount of time spent in the compartment during training. Each rat was confined in the drug-paired compartment for 20 minutes a day for four days, which may not have been a sufficient amount of time to develop a preference. Zhu et al. (2011) confined rats in the CPP apparatus for 30 minutes twice a day for five days; Wooters et al. (2011) confined rats for 30 minutes a day for eight days; and Sellings, McQuade, and Clarke (2006) confined rats for 15 minutes a day for six days. The rats spent more time in the drug-paired compartment in these studies during training and developed a conditioned preference.

Problems with the design of the study that may have contributed to the unexpected results are the biased design and the distinctions between the compartments. The design of the study was biased in that the rats were adapted to the CPP apparatus to measure a baseline preference. The drug-paired and non-drug paired compartments were based on the baseline preference rather than being randomly assigned. This was done to ensure drugs would be paired with the non-preferred compartment; and any conditioned preference could be attributed to the drug alone. The rats had already established a preference, which could have influenced their preference during testing. Although a biased design could have influenced the conditioned preference, it is a widely accepted variation of the CPP paradigm. The distinctions between the drug-paired and non-drug paired compartments could have also contributed to the unexpected results. The non-drug paired compartment had black walls and a wood floor with bedding on top, and the drug-paired compartment had white walls and a wood floor with no bedding on top. The compartments could not be identical because the distinctions acted as environmental cues for conditioned stimuli. Rats innately prefer dark rooms and bedding over light rooms. Having two innate preferences in the same compartment could have influenced the rat's decision to spend more time in that compartment. This study should have made the compartments equally appealing with small differences to decrease the bias of always preferring the dark room and bedding. For example, the compartments could have had the same color walls with a different texture on the floors or they could have had the same floor with vertical stripes in one compartment and horizontal stripes in the other compartment. This would have prevented the rat's innate preferences from influencing the conditioned preference. Although there could have been better distinctions between compartments and a non-biased design, the study was a between subjects design that avoided any carryover effects from other treatment groups.

Future studies could determine the effects of Clozapine on a variety of different doses of MPH. This could determine if the abuse potential is dose-dependent and what doses have the strongest rewarding effect. Researchers should also ensure a non-baised design of their study and have equally appealing compartments to decrease any innate preferences that may influence the rat's conditioned preference. In addition to testing Clozapine with other MPH doses, future studies could analyze dopamine levels in the striatum and prefrontal cortex using TH Staining or High-Performance Liquid Chromatography (HPLC). This would show a more definitive effect of Clozapine on MPH. The exact DA levels could be determined and compared to the results of the CPP tests to see if they are consistent with each other. If it is shown through multiple trials that Clozapine inhibits MPH, further testing can be done to use Clozapine concurrently with MPH to decrease its abuse potential. Lastly, if this is established, testing can be done to find a similar drug to Clozapine without its many aversive side effects.

In summary, this study showed that MPH10 and MPH5 both produced an aversion to the drug-paired compartment rather than the predicted conditioned preference. Also, it can not be implied that Clozapine produced an aversion due to its inhibiting effects on MPH10 because MPH10 by itself also produced an aversion. It will be important to retest MPH10 and MPH5 to determine if the distinctions between the compartments influenced the aversion to the drug-paired compartment. This study conflicted with other studies in that they were able to produce a conditioned preference to the drug-paired compartment. If it is established that the disctinctions influenced their preference, more testing will be needed to replicate the predicted conditioned preference to the drug-paired compartment.

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