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Antagonizing Serotonin 2A (5-HT2A) Receptors Does Not Attenuate Methamphetamine-Induced Expression of Conditioned Place Preference in Female and Male Rats

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by

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ABSTRACT

Despite the need, there are currently no FDA-approved pharmacological treatments for methamphetamine use disorders. Cue-induced craving during drug withdrawal remains a significant contributor to high relapse rates. Thus, understanding the neurobiological mechanisms of cue-induced drug-seeking behavior is critical. Previous research indicates that the selective 5-HT_{2A} antagonist M100907 attenuates the acquisition of methamphetamine-induced conditioned place preference (CPP) in male and female adult rats. The present study examines the effects of M100907 on expression of methamphetamine-induced drug-seeking behavior using a biased CPP design in adult male and female Long Evans rats (PND 95-112). During conditioning, rats were administered either saline or methamphetamine (1 mg/kg/ml, i.p.) and immediately placed into their initially non-preferred chamber. Rats received either vehicle or M100907 (0.0025, 0.025, 0.1 mg/kg i.p.) 15 minutes prior to expression testing. We found that none of the M100907 doses significantly altered the expression of methamphetamine-induced CPP in male nor female rats.

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1. Introduction

Methamphetamine use continues to impact society, with approximately 1.6 million adults using methamphetamine every year and 50% of those who use methamphetamine meeting the diagnostic criteria for methamphetamine use disorder (MUD; Jones, 2020). MUD is a growing problem that contributes significantly to individual health-related morbidity such as cardiovascular and renal problems, mental disturbances and psychosis (Jones, 2020; Courtney & Ray, 2014; Jayanthi et al., 2021). The impact of methamphetamine abuse extends beyond individual harm, contributing to societal issues including crime, unemployment, and child neglect (NIDA, 2021). Despite the sharply increasing prevalence of MUD, especially in minority communities, there are no FDA-approved medications for treating MUDs or reversing drug overdoses (Han, 2021), highlighting the need for research examining novel pharmacotherapies.

Chronic use of psychostimulants such as methamphetamine results in physical dependence, leading to a cluster of withdrawal symptoms consisting of sleep and appetite disturbances, fatigue, depressed mood, irritability, heightened stress reactivity, drug craving and anxiety (Darke et al., 2008; Zhao, 2021; Sanchez-Ramos, 2015). Due to the unpleasantness of these withdrawal symptoms, relapse is common, with 61% of abstinent patients relapsing to methamphetamine use within a year of treatment (Brecht & Herbeck, 2014). Thus, current literature urges for a combination of psychosocial therapies and pharmacotherapy for alleviation of withdrawal symptoms (Bhatt et al., 2016).

Recent evidence implicates the serotonin 2A (5-HT_{2A}) receptor subtype in the behavioral consequences of psychostimulants, therefore making it a promising target for treating psychostimulant use disorders. Several studies have reported 5-HT_{2A} receptor antagonists decrease hyperlocomotive effects of cocaine, methamphetamine and 3,4-Methylenedioxy

methamphetamine (MDMA) in male rats (Bankson & Cunningham, 2002; Bubar & Cunningham, 2006; Bubar & Cunningham, 2008; Herin et al., 2005). Studies examining cocaine indicate that administration of non-selective 5-HT_{2A} receptor antagonists depresses cocaine cue-induced reinstatement and blocks acquisition and expression of single-trial cocaine CPP in male rats (Bubar & Cunningham, 2006; Bubar & Cunningham, 2008). M100907, a selective 5-HT_{2A} receptor antagonist, has been reported to have more robust effects, with both acquisition and expression of cocaine-induced CPP blocked via pretreatment with M100907 in male rats (dela Cruz, 2009). Additionally, M100907 suppresses both cocaine-primed and cue-evoked reinstatement in the self-administration paradigm in both male rats and non-human primates (Nic Dhonnchadha et al., 2009; Murnane et al., 2013). These effects likely result from altered mesolimbic function, as M100907 attenuates cocaine-induced overflow in the caudate nucleus in non-human primates, thereby reducing mesocortical dopamine release which plays a critical role in drug- and cue-induced reinstatement (Murnane et al., 2013). M100907 has also been implicated in modulating nicotine-induced behaviors. One study reported that M100907 reduces nicotine-primed or cue-induced reinstatement in male rats (Fletcher et al., 2012). Another study indicated that M100907 significantly reduces the depression-like behavioral effects in male rats during nicotine withdrawal (Zaniewska et al., 2010). Additionally, M100907 reduces cocaine- and amphetamine-induced impulsive actions in male rats (Fletcher et al., 2002). Collectively, these data ascribe a critical role for 5-HT_{2A} receptors in regulating the behavioral effects of psychostimulants.

Studies examining methamphetamine specifically report that M100907 attenuates acquisition of methamphetamine-induced reward in male and female rats in the CPP paradigm (Madden et al., 2020; 2021) and decreases anxiety-like behavior following chronic

methamphetamine exposure in male rats (Madden et al., 2021). Additionally, M100907 dose-dependently decreases methamphetamine self-administration and methamphetamine-seeking behavior in male and female rhesus macaques (Odabas-Geldiay et al., 2019) and male rats (Graves et al., 2012). Chronic methamphetamine exposure causes dysregulation of 5-HT_{2A} receptors in the medial prefrontal cortex (PFC) and perirhinal cortex, leading to increases in 5-HT_{2A} receptor expression in male rats (Hámor et al., 2018). Additionally, chronic methamphetamine increases 5-HT_{2A} receptors in the PFC and ventral tegmental area (VTA), leading to increased levels of dopamine neurons in mesocorticolimbic structures in male rats (Doherty & Pickel, 2000; Alex & Pehek, 2007). Thus, blocking 5-HT_{2A} with M100907 may dampen this heighted dopamine signaling, leading to attenuation of drug-seeking behavior and drug reward (Cunningham et al., 2013; Pehek et al., 2006).

In the present study, we hypothesized that blocking 5-HT_{2A} receptors will dose-dependently attenuate expression of methamphetamine-induced CPP in both male and female rats. A substantial proportion of pyramidal neurons in the PFC that project to areas such as the dorsal raphe and VTA express higher concentrations of 5-HT_{2A} receptors compared to other 5-HT receptors (Vázquez-Borsetti et al., 2009). These areas are significant for neurological disorders, such as addiction, because they contain high levels of serotonergic innervation (Mengod et al., 2015), and activation of these receptors leads to increased mesolimbic dopamine release. Females exhibit increased 5-HT receptors expression throughout the entire brain, with increased levels in critical addiction-related structures such as the striatum and cortex (Carlsson & Carlsson, 1988; Summer & Fink, 1995). However, studies have reported that females have diminished binding to the postsynaptic 5-HT_{2A} receptors compared to males (Biver et al., 1996;

Soloff et al., 2010), which likely mitigates these sex differences in the serotonergic system and diminishes the likelihood that any sex differences would impact the present study.

2. Materials and methods

2.1. Animals

Subjects were adult female and male Long-Evans hooded rats (N=94, Male N=46, Female N=48; 97-111 days old), born and reared in the Logan Hall facility at the University of New Mexico. All rats were pair housed in standard home cages (21.6 x 45.7 x 17.8 cm) in a temperature-controlled colony room (21-24 ◦C) with a reverse 12:12 light/dark cycle (lights off at 10 am), with food and water available *ad libitum* in their home cages. Rats were handled for one week prior to testing. All husbandry and experimental procedures adhere to the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and were approved by the University of New Mexico Institutional Animal Care and Use Committee.

2.2. Drugs

M100907 (Axon Medchem LLC, Reston, VA, USA) and methamphetamine hydrochloride (Sigma Aldrich, St. Louis, MO, USA) were dissolved in 0.9% sterile saline and administered intraperitoneally (i.p.) at a volume of 1 ml/kg of body weight; M100907 also contained 3% Tween 20 (vehicle). The doses of methamphetamine (0 or 1 mg/kg, i.p.) and M100907 (0, 0.0025, 0.025, 0.1 mg/kg, i.p.) were selected based on previous research showing that this methamphetamine dose is effective in producing reliable CPP and that these doses of M100907 attenuate methamphetamine-induced hyperactivity and acquisition of methamphetamine-induced CPP (Zakharova et al., 2009; Steed et al., 2011; Madden et al., 2020).

2.2.1 Behavioral testing

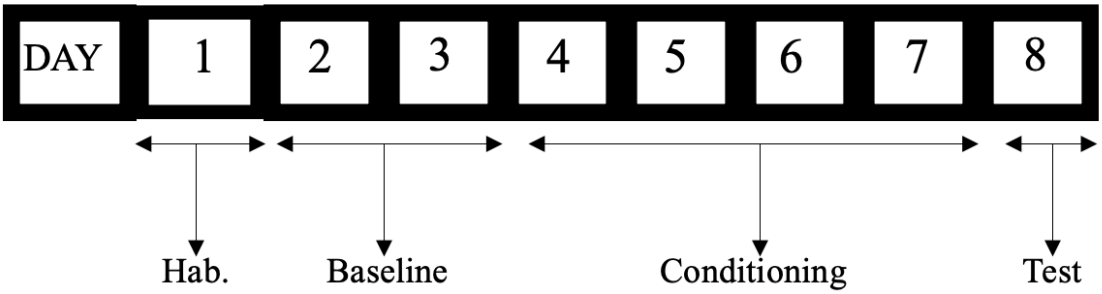
2.2.2. Conditioned place preference

Conditioning was conducted in plexiglass apparatuses each composed of two equal-sized compartments (25 x 25 x 45 cm). The two conditioning compartments were divided by a removable solid partition. On habituation, baseline and preference test days the partition was removed and replaced with a partition that contained a small opening (8 x 8 cm), allowing free access to both compartments. The two conditioning compartments are distinguishable by color, odor and texture. Specifically, one compartment consists of a grid flooring, corncob bedding and white walls while the other compartment consists of bar flooring, pine bedding and black walls.

The first day, designated as habituation, consisted of each rat being placed into the apparatus and allowed to freely explore both compartments for 15 minutes in order to acclimate to the novel environment. The time spent in each compartment was not recorded on this day. The next two days, designated as baseline, were identical except the amount of time the rats spent in each side of the apparatus was recorded and averaged across the two days in order to determine the rats' initially non-preferred side, operationally defined as the compartment in which the rats spent less than 50% of their total time. Overall, both males and females showed a preference for the black/bar side compared to the white/grid side (see Tables 1 and 2), suggesting that the apparatus was biased. However, the means for each non-preferred side were nearly equal and the experimental groups were counterbalanced for baseline preference across both compartments. Conditioning occurred over the next four days and consisted of two thirty-minute sessions per day, with the first session occurring in the morning and the second session occurring four hours later to allow for drug clearance. This study utilized a biased design where methamphetamine was paired with the rats' initially non-preferred side. During the conditioning sessions, rats in all groups received a saline injection prior to being placed in their preferred side. The order of saline/methamphetamine injections was counterbalanced each day of conditioning to control for

potential effects of time of day. On the final day of testing (i.e., expression testing), rats were administered their assigned dose of M100907 (0.0025, .025, 0.1) or saline 15 minutes prior to being placed in the CPP box. The amount of time the rats spent on each side was recorded. (See Figure A for experimental timeline). Throughout all phases of the CPP procedure, a continuous white noise generator was set at 15 dB above background to minimize ambient noise and enhance the salience of the environment. All sessions were conducted under red light and all apparatuses used were promptly wiped down with 5% ethanol after each trial.

Figure A: Timeline



Experimental design and timeline of the 8-day conditioned place preference (CPP) procedure. The experiment consisted of 1 day of habituation (Hab.), 2 days of baseline preference testing, 4 days of conditioning (8 conditioning sessions total/ 2 a day), 1 expression test trial. The habituation, baseline, and test were 15 minutes. During baseline days (2-3), rats were allowed to freely roam the two compartments and initial side preference was recorded. For conditioning days (4-7), rats received methamphetamine (1.0 mg/kg) on their non-preferred side and saline on their preferred. Each conditioning session was 30 minutes and occurred twice a day using a counterbalanced design. One preference testing day (8) occurred following the last day of conditioning to assess the rats' final preference.

2.3. Data Analysis

Methamphetamine-induced CPP was operationally defined as a significant increase in time spent on the initially non-preferred side (i.e., baseline) following conditioning. In order to ensure there were no strong deviations in initial preference, a one-way ANOVA comparing the baselines for each group was conducted. To examine the magnitude of M100907 effects on

methamphetamine-induced CPP, group differences were examined using both difference scores and percent change from baseline scores. Difference scores were calculated by taking the time spent in the non-preferred side during the expression test minus the time spent in the non-preferred side during initial baseline testing. Percent change from baseline scores were calculated using the formula [(A2-A1)/A1] *100, where A2 represents time spent in the non-preferred side during the expression test and A1 represents the time spent in the non-preferred side during baseline testing. In order to verify that the dose of methamphetamine was rewarding (i.e., produced CPP), separate independent samples t-tests were run comparing control (Saline-Tween) and methamphetamine (Meth-Tween) groups on measures of percent change and difference from baseline (i.e., test – baseline). Within group CPP was also determined for the control (Saline-Tween) and methamphetamine (Meth-Tween) groups using paired samples t-tests. To examine the effects of M100907 on expression of methamphetamine-induced CPP, potential differences in time spent on the drug-paired side on baseline and test day was analyzed using a repeated measures ANOVA with M100907 dose as the between group variable (Meth-M0.0025, Meth-M0.025, Meth-M0.1) and baseline and test day as within factors. In order to fully characterize the effects of each dose of M100907 on expression of methamphetamine-induced CPP, potential differences in preference on baseline and test day were probed using paired sample t-tests for each M100907 group (Meth-M0.0025, Meth-M0.025, Meth-M0.1); potential group differences in percent change were also analyzed using a one-way ANOVA.

3. Results

3.1 Males

Figure 1 depicts methamphetamine-induced CPP in males. A one-way ANOVA comparing group baselines prior to conditioning was not significant [$F(4, 39) = 0.492$, $p = 0.741$],

indicating that there were no group differences in the magnitude of their initial baseline preference (Figure 1). An independent samples t-test examining percent change found that methamphetamine was rewarding in males (i.e., produced CPP) with the Meth-Tween group (M= 49.18, SD=21.98) showing a significantly larger percent change (Figure 1B; $t(15)= -5.00$, $p<.001$) compared to Saline-Tween group (M= -3.45, SD= 21.25). Similarly, an independent samples t-test investigating difference scores (i.e., post-conditioning change in baseline preference) confirmed that methamphetamine produced CPP with Meth-Tween (M=187, SD=57.22) rats exhibiting significantly larger difference scores (Figure 1C; $t(15)=-5.92$, $p<.001$) compared to Saline-Tween (M=-16.56, SD= 83.60) rats. Lastly, paired samples t-tests indicated that the Meth-Tween ($t(8)=-9.80$, $p<.001$) group but not the Saline-Tween ($t(7)=0.560$, $p=0.593$) group exhibited a significant preference switch following conditioning.

Figure 2 illustrates the effects of M100907 on expression of methamphetamine-induced CPP. The repeated measures ANOVA testing the impact of M100907 on methamphetamine-induced CPP (Meth-Tween, Meth-M0.0025, Meth-M0.025, Meth-M0.1) detected a significant main effect of day ($F(1,28) = 160.67$, $p<0.001$), but not a day by M100907 interaction ($F(3, 28) = 1.69$, $p=0.193$). This suggests that M100907 did not alter expression of methamphetamine-induced CPP. To further probe for potential M100907 dose effects, paired samples t-test were conducted for each M100907 group (Meth-M0.0025, Meth-M0.025, Meth-M0.1). Rats in the Meth-M0.0025 ($t(7)=-5.56$, $p<.001$), Meth-M0.025 ($t(7)=-4.22$, $p=.004$) and Meth-M0.1 ($t(8)=-4.38$, $p=0.002$) groups all showed a significant preference shift following methamphetamine conditioning. Lastly, we compared percent change and difference scores using a one-way ANOVA. Neither percent change (Figure 2A; $F(3,30)=0.132$, $p=0.940$) nor difference scores (Figure 2B; $F(3,30)=0.049$, $p=0.985$) showed a significant difference between methamphetamine

groups. Collectively, these data indicate that methamphetamine reliably induced expression of CPP, but M100907 did not attenuate the rewarding effects of methamphetamine during expression testing.

Table 1: Male preconditioning baseline preferences

Non-preferred side	#	Percent of rats	Black Time ± SD	White Time ± SD
Black/Bar	16	36.4%	**392.28 ± 39.66**	507.5 ± 39.49
White/Grid	28	63.6%	502.64 ± 46.79	**397.36 ± 46.79**
Total	44	100%	462.51 ± 69.33	437.41 ± 69.22

Baselines were computed as an average of the two pre-conditioning sessions. **Bold** numbers indicate the non-preferred compartment. Methamphetamine was paired with the **bolded**/non-preferred side of the CPP box during conditioning.

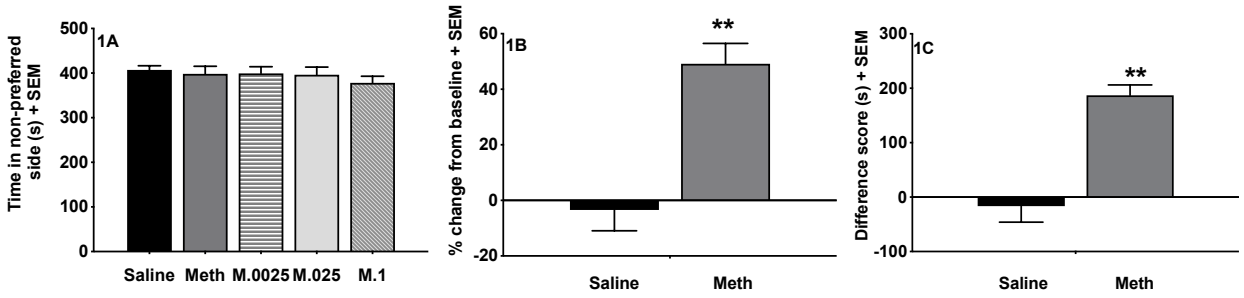


Figure 1: Methamphetamine produced robust CPP in males
A one-way ANOVA indicated that rats in each experimental group did not differ in the amount of time spent in their initially non preferred side prior to methamphetamine (Meth) conditioning [Figure 1A; $F(4, 39) = 0.492$, $p = 0.741$]. Following conditioning, Meth treated rats showed a strong conditioned place preference with a significantly larger percent change from baseline (Figure 1B; $t(15) = -5.00$, $p < .001$) and a significantly larger difference score (Figure 1C; $t(15) = -5.92$, $p < .001$) compared to saline controls. Asterisks (**) represent a significant difference compared to controls ($p < 0.001$).

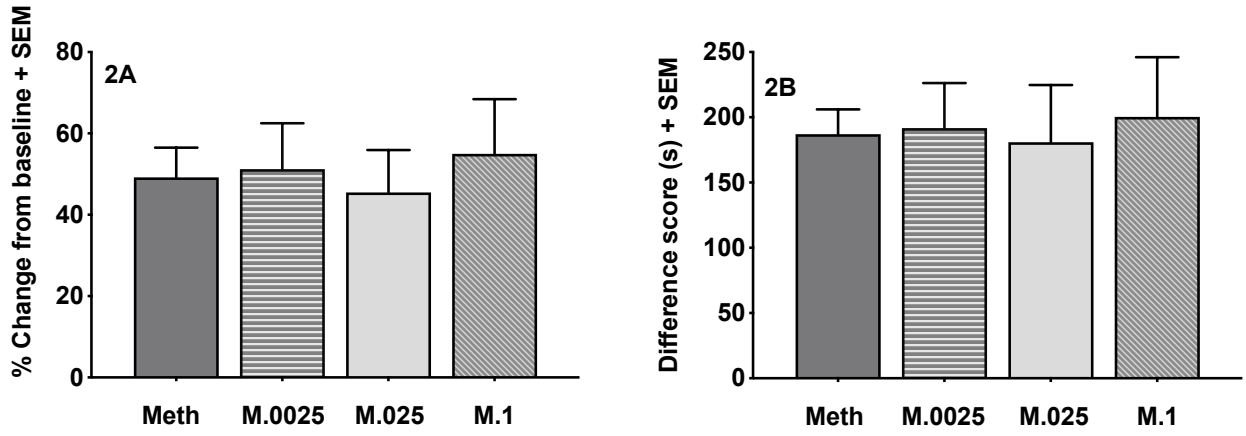


Figure 2: Effects of M100907 on methamphetamine in males
M100907 failed to alter expression of methamphetamine (Meth)-induced conditioned place preference (CPP). Following conditioning, rats received their assigned dose of M100907 (Meth-Tween, Meth-M0.0025, Meth-M0.025, and Meth-M0.1) and then were tested for CPP. All Meth groups demonstrated a significant preference shift following methamphetamine conditioning with a one-way ANOVA failing to detect significant group differences in % change from baseline [Figure 2A; $F(3,30)=0.132$, $p=0.940$] or significant difference scores [Figure 2B: $F(3,30)=0.049$, $p=0.985$].

3.1.2 Females

Figure 3 depicts methamphetamine-induced CPP in females. A one-way ANOVA comparing baselines prior to conditioning was not significant [$F(4, 43) = 0.086$, $p = 0.986$], indicating that there were no group differences in the magnitude of their initial baseline preference (Figure 3A). An independent samples t-test examining percent change found that the dose of methamphetamine was rewarding in females (i.e., produced CPP), with the Meth-Tween group ($M= 43.66$, $SD= 26.16$) showing a significantly larger percent change (Figure 3B; $t(18)= -2.61$, $p=0.009$) compared to the Saline-Tween group ($M= 12.27$, $SD= 24.55$). Similarly, an independent samples t-test investigating difference scores (i.e., post-conditioning change in baseline preference) confirmed that methamphetamine produced CPP with Meth-Tween rats exhibiting significantly larger group differences (Figure 3C; $t(18)= -2.81$, $p= 0.006$) compared to Saline-Tween rats. Lastly, paired samples t-tests indicated that the Meth-Tween ($t(12)= -6.39$,

p<0.001) group but not the Saline-Tween (t(6)= -1.22, p=0.134) group exhibited a significant preference switch following conditioning.

Figure 4 illustrates the effects of M100907 on expression of methamphetamine-induced CPP. The repeated measures ANOVA testing the effects of M100907 on methamphetamine-induced CPP (Meth-Tween, Meth-M0.0025, Meth-M0.025, Meth-M0.1) detected a significant main effect of day (F (1,35) =184.60, p<0.001), but not a day by M100907 group interaction (F(3, 35)=1.12, p=0.354). This suggests that M100907 did not alter expression of methamphetamine-induced CPP for females. To further probe for potential M100907 dose effects, paired samples t-tests were conducted for each M100907 group. Rats in the Meth-M0.0025 (t(7)= -6.88, p<0.001), Meth-M0.025 (t (8)=-5.56, p<0.001) and Meth-M0.1 (t (10)=-5.02, p<0.001) groups all showed a significant preference shift following methamphetamine conditioning. Lastly, we compared percent change and difference scores using a one-way ANOVA and found no significant differences between methamphetamine groups respectively (Figure 4A; F(3,35)=0.851, p=0.476); Figure 4B; F(3,35)=1.12, p=0.354). Collectively, these data indicate that methamphetamine reliably induced expression of methamphetamine-induced CPP, but M100907 did not attenuate the rewarding effects of methamphetamine in females during expression testing.

Table 2: Female preconditioning baseline preferences

Non-preferred side	#	Percent of rats	Black Time ± SD	White Time ± SD
Black/Bar	18	37.5%	**418.25 ± 28.22**	481.75 ± 28.22
White/Grid	30	62.5%	509.33 ± 40.62	**390.67 ± 40.62**
Total	48	100%	475.18 ± 57.37	424.82 ± 57.37

Baselines were computed as an average of the two pre-conditioning sessions. **Bold** numbers indicate the non-preferred compartment. Methamphetamine was paired with the **bolded**/non-preferred side of the CPP box during conditioning days.

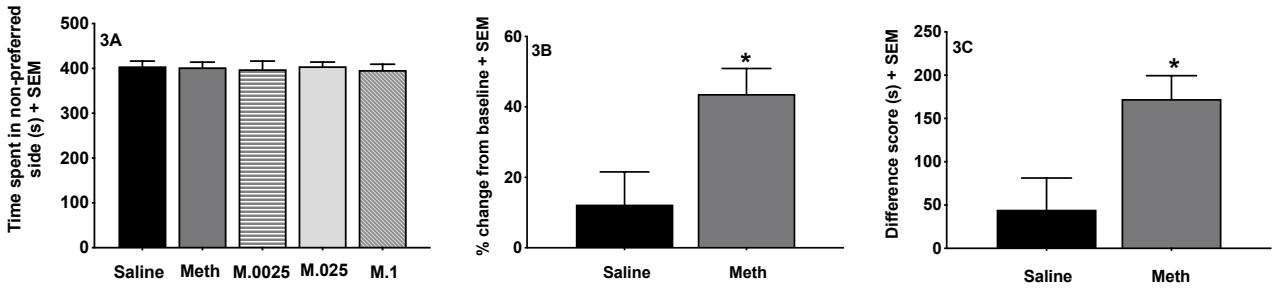


Figure 3: Methamphetamine produced robust CPP in females
A one-way ANOVA indicated that rats in each experimental group did not differ in the amount of time spent in their initially non preferred side prior to methamphetamine (Meth) conditioning [Figure 3A; [F (4, 43) = 0.086, p = 0.986]. Following conditioning, Meth treated rats showed a strong conditioned place preference with a significantly larger percent change from baseline (Figure 3B; t(18)= -2.61, p=0.009) and a significantly larger difference score (Figure 3C; t t(18)= -2.81, p= 0.006) compared to saline controls. Asterisks (*) represent a significant difference compared to controls ($p<0.05$).

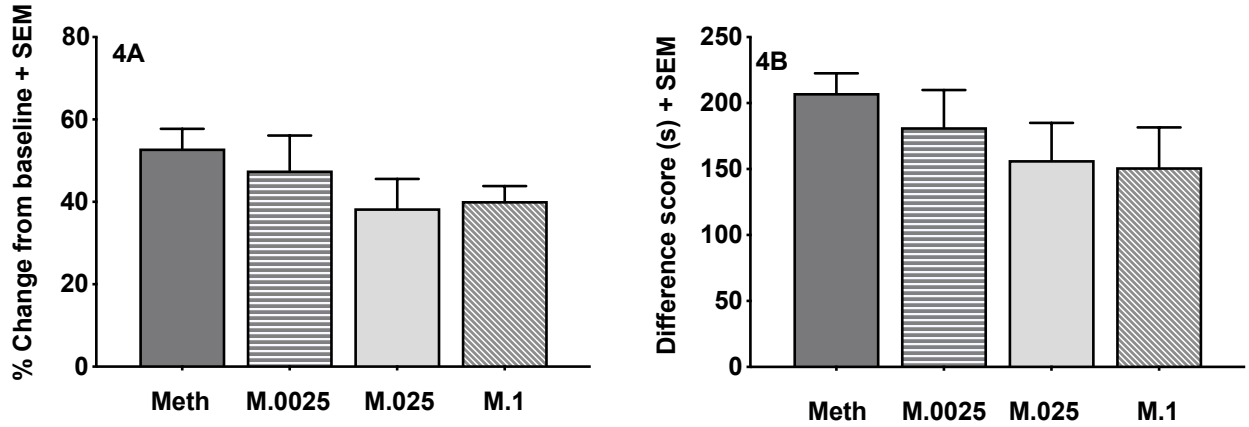


Figure 4: Effects of M100907 on methamphetamine in females
M100907 failed to alter expression of methamphetamine (Meth)-induced conditioned place preference (CPP). Following conditioning, rats received their assigned dose of M100907 (Meth-Tween, Meth-M0.0025, Meth-M0.025, and Meth-M0.1) and then were tested for CPP. All Meth groups demonstrated a significant preference shift following methamphetamine conditioning with a one-way ANOVA failing to detect significant group differences in % change from baseline [Figure 4A; F(3,35)=0.851, p=0.476] or significant difference scores [Figure 4B: F(3,35)=1.12, p=0.354].

4. Discussion

Results from the present study indicate that M100907 (0.0025 – 0.1 mg/kg) did not attenuate expression of methamphetamine-induced CPP. In male rats, there were no significant differences in baseline preference between groups (Figure 1A), and methamphetamine produced robust reward (Figure 1B, 1C). In contrast to our hypothesis, administration of M100907 prior to expression testing did not significantly alter methamphetamine-induced CPP in males (Figure 2A, 2B). Similarly, in females there were no significant group differences in initial baseline preference (Figure 3A), methamphetamine reliably produced reward (Figure 3B, 3C), and M100907 did not decrease time spent in the methamphetamine paired side at any of the three doses of M100907 tested for female rats (Figure 4A, 4B). Although we initially hypothesized that there would be a dose-dependent effect, none of the M100907 doses significantly altered the expression of methamphetamine-induced CPP. Collectively, these data along with previous findings indicate that blocking 5-HT_{2A} receptors with M100907 attenuates acquisition (Madden et al., 2020; 2021) but not expression of methamphetamine-induced CPP in adult male and female rats.

One potential reason we failed to detect effects of M100907 on expression of methamphetamine-induced CPP despite previous research reporting an attenuating effect on acquisition of methamphetamine-induced CPP (Madden et al., 2020; 2021) could be that M100907 affects spontaneous locomotion. In the previous reports by Madden and colleagues, rats did not have methamphetamine or M100907 onboard during expression testing, while in the current study rats received M100907 injections before expression testing. Although not directly tested in the present study, previous research suggests that M100907 alters stimulant-induced but not spontaneous locomotion. For example, M100907 reduces cocaine-induced hyperlocomotion (0.05 mg/kg; Pockros et al., 2012) and inhibits amphetamine-induced hyperlocomotion without

affecting spontaneous locomotion (0.1 – 3.0 mg/kg; Moser et al., 1995). Similarly, Zaniewska and colleagues (0.5-2.0 mg/kg) and McMahon and colleagues (0.02 – 2.0 mg/kg) report that M100907 does not change spontaneous locomotor activity across a wide range of doses (Zaniewska et al., 2009; McMahon & Cunningham, 2001). Although the doses in the previous studies vary slightly, collectively they suggest that the doses of M100907 used in the present study likely had no impact on spontaneous locomotion and mitigate impaired movement as a reason for the null effects of M100907 on expression of methamphetamine-induced CPP in the present study.

Studies examining the effects of M100907 on psychostimulant reinforcement generally fail to find significant modulatory effects. Indeed, M100907 (0.5-2.0 mg/kg) did not attenuate cocaine or nicotine self-administration (Fletcher et al., 2002; 2012; Bubar & Cunningham, 2008). These findings suggest that M100907 fails to alter reinforcement of psychostimulants. However, two primate studies examining the effects of 5-HT_{2A} antagonism on methamphetamine self-administration suggest that M100907 may modulate stimulant reinforcement. Banks and colleagues report that the inverse 5-HT_{2A} receptor agonist Pimavanserin failed to attenuate methamphetamine reinforcement in rhesus macaques (Banks et al., 2016), while Odabas-Geliday and colleagues reported that M100907 attenuates methamphetamine reinforcement in rhesus macaques (Odabas-Geliday et al., 2019). There are several reasons why these two studies report conflicting results. First, each study was underpowered, using only 3 nonhuman primates, which could somewhat limit firm conclusions regarding behavioral outcomes. Second, different 5-HT_{2A} drugs and/or different drug doses may have led to different results. Indeed, Pimavanserin doses were 1.0-10 mg/kg while M100907 doses were 0.03-0.3 mg/kg (Banks et al., 2016; Odabas-Geliday et al., 2019). Although the two drugs are both 5-HT_{2A} antagonists, the drugs differ in

receptor selectivity, with Pimavanserin only having a 40-fold selectivity for the 5-HT_{2A} receptor over the 5-HT_{2C} receptor while M100907 has 100-fold selectivity for the 5-HT_{2A} receptor over the 5-HT_{2C} receptor. Despite these differences, Pimavanserin and M100907 both elicit similar behavioral effects. For instance, both Pimavanserin and M100907 have been reported to reduce psychotic symptoms in both humans and rats (McFarland et al., 2011). Collectively, these data suggest that blocking 5-HT_{2A} receptors generally fails to alter psychostimulant reinforcement.

When examining results from the CPP paradigm specifically, the present findings seem to conflict with previous research indicating that M100907 attenuates acquisition of CPP in male and female rats (Madden et al., 2020; 2021). However, there are two important differences that could account for these differing outcomes. First, although each of the studies used female and male Long-Evans hooded rats, the doses of M100907 varied slightly. The previous study used an M100907 dose range of 0.025-0.25 mg/kg (Madden et al., 2020; 2021) while the present study used an M100907 dose range of 0.0025-0.1 mg/kg. Perhaps, a broader range of doses might alter expression of methamphetamine-induced CPP similar to acquisition. Second, the present study examined expression of methamphetamine-induced CPP by giving rats an acute injection of M100907 prior to the expression test. In contrast, in Madden and colleagues' study, rats received M100907 prior to each of the four methamphetamine conditioning sessions. Therefore, the effects of M100907 during the acquisition phase indicate that blocking 5-HT_{2A} receptors either reduces the subjective reward experience or prevents the conditioned association of the rewarding experience with the distinct context of the CPP apparatus. The current study examined if M100907 would also attenuate reward seeking following methamphetamine conditioning. Therefore, it is possible that M100907 is effective at blocking the reward-environment pairing experience, but not effective at decreasing drug seeking after this reward-environment pairing

has already been established. However, other research examining psychostimulant seeking in rats found that various doses of M100907 attenuate drug seeking. For example, M100907 (0.5-2.0 mg/kg) attenuates cue-induced and nicotine-primed reinstatement of extinguished nicotine-seeking behavior (Fletcher et al., 2012) and M100907 (0.001-0.8 mg/kg) suppresses cue-induced reinstatement of extinguished cocaine-seeking behavior (Nic Dhonnchadha et al., 2009). A study establishing subthreshold doses for M100907 on drug seeking reported different outcomes depending on the behavioral measure. For example, when examining cocaine-primed reinstatement, the subthreshold dose of M100907 is 0.1 mg/kg, while the effective dose range of M100907 when examining cue-induced cocaine reinstatement is 0.001 mg/kg-0.01 mg/kg (Cunningham et al., 2013). Collectively, these data examining the effects of blocking 5-HT_{2A} receptors on drug-seeking behavior suggest that higher doses of M100907 may be needed to alter expression of methamphetamine-induced CPP.

Although in the introduction we stated that chronic methamphetamine exposure causes increased 5-HT_{2A} receptor expression in the PFC of male rats, Hámor and colleagues note that this may be the result of a compensatory response to initial serotonin depletion following methamphetamine use (Hámor et al., 2018). Neurobiologically, chronic methamphetamine exposure leads to decreased frontal cortical serotonin levels in rodents (Heal et al., 1985; Mcfadden et al., 2013). Rats chronically administered d-amphetamine followed by a 24-hour withdrawal period show significantly decreased 5-HT_{2A} receptor mRNA expression in the prefrontal, motor and cingulate cortices, while 5-HT_{2A} receptor expression is increased in the NAc, caudate putamen (CPu) and hippocampus (Horner et al., 2011). The authors speculate that this may be due to the body's compensatory response to amphetamine-induced serotonin influx, with the decreased activation of the PFC leading to decreased activation of downstream brain

structures such as the NAc, CPu and hippocampus. In order to compensate, this leads to an increase in 5-HT_{2A} receptor expression in the NAc, CPu, and hippocampus (Horner et al., 2011). Depletion of serotonin in frontal cortical structures leads to increased impulsivity, which could exacerbate the likelihood of relapse (Harrison et al., 1997). M100907 has been shown to increase attentional performance and reduce impulsivity in rats (Winstanley et al., 2003). Additionally, M100907 decreases cocaine-induced Fos protein expression in the CPu (Pockros et al., 2012). This suggests that although there is decreased serotonin receptor expression in the PFC, M100907 may still help attenuate methamphetamine-induced reward, given M100907s efficaciousness at attenuating impulsivity and Fos expression. This could further explain why Madden and colleagues saw attenuation in acquisition of methamphetamine-induced CPP. M100907 was administered prior to methamphetamine administration, suggesting that M100907 could block the serotonergic influx caused by methamphetamine. This could make methamphetamine less rewarding and account for the decrease in time spent on the methamphetamine paired side of the chamber on test day. However, in the current study, M100907 was not administered with methamphetamine; therefore, it is possible the serotonergic system was already depleted when testing occurred. Thus, M100907 would not have any impact on expression of methamphetamine-induced CPP, as reported in the current study. Additionally, levels of PFC 5-HT_{2A} receptors is critical, with a study reporting that the activity and release of dopamine neurons in the VTA and PFC are modulated by PFC 5-HT_{2A} receptors (Bortolozzi et al., 2005). Although administration of a 5-HT_{2A} receptor antagonist does not alter tonic firing rates of VTA dopamine neurons, they do reverse the increase in phasic dopamine firing evoked by 5-HT_{2A} receptor agonists (Bubar & Cunningham, 2008; Pehek et al., 2006). It has also been shown that administration of M100907 blocks psychostimulant-induced increases in dopamine

release in the VTA (Pehek et al., 2001). Collectively, downregulation of 5-HT_{2A} receptors following chronic methamphetamine exposure may explain why rats in the present study did not respond to 5-HT_{2A} receptor antagonism and still exhibited drug-seeking behavior.

Another important receptor to consider that regulates the mesolimbic dopamine pathway is the 5-HT_{2C} receptor (Alex & Pehek, 2007). Indeed, 5-HT_{2C} receptor agonists decrease basal firing rates of VTA dopamine neurons and dopamine efflux into the NAc (Bubar & Cunningham, 2008; De Deurwaerdère & Spampinato, 1999). Systemic administration of a 5-HT_{2C} receptor agonist results in inhibition of mesolimbic dopamine activity and suppression of THC- and nicotine-induced CPP in rats (Ji et al., 2006; Alex and Pehek, 2007). Additionally, systemic administration of the 5-HT_{2C} agonist R0-60175 leads to a decrease in dopamine efflux in the PFC and blocks stress-induced dopamine increases in the PFC (Alex and Pehek, 2007). Importantly, the 5-HT_{2C} agonist MK212 blocks expression of cocaine-induced CPP in male rats (dela Cruz et al., 2009). Together, these data suggest that a combination of a 5-HT_{2A} receptor antagonist and a 5-HT_{2C} receptor agonist may elicit more potent behavioral responses, in contrast with the present study which only used one pharmacological intervention.

Due to the complexity of the serotonergic system and prominent roles of the 5-HT_{2A} and 5-HT_{2C} receptors, future studies should investigate a combination of pharmacotherapies, specifically a 5-HT_{2C} receptor agonist and 5-HT_{2A} receptor antagonist. Studies report that antagonizing the 5-HT_{2A} receptor and agonizing the 5-HT_{2C} receptor blocks the discriminative stimulus effects of cocaine and nicotine (Bubar & Cunningham, 2008; McMahon & Cunningham., 2001; Zaniewska et al., 2009), and administration of a 5-HT_{2C} receptor agonist and a 5-HT_{2A} receptor antagonist blocks acquisition and expression of cocaine-induced CPP in rats (dela Cruz et al., 2009). Additionally, both M100907 and the 5-HT_{2C} receptor agonist

MK212 synergistically attenuate cocaine-induced dopamine release and hyperlocomotion (Pockros et al.,2012). Further, M100907 and the 5-HT_{2C} receptor agonist WAY163909 synergistically suppress cocaine hyperactivity as well as cue- and cocaine-primed reinstatement of cocaine seeking in rats (Cunningham et al., 2013). Acute 5-HT_{2C} agonist (Lorcaserin) administration decreases cocaine self-administration, while treatment with Lorcaserin plus Pimavanserin decreases cocaine seeking in rats (Anastasio et al., 2020). Collectively, these data suggest that combinations of pharmacotherapies targeting the 5-HT_{2A} and 5-HT_{2C} receptor families could be more effective in treating both substance use disorder in general and MUD specifically.

There is also clinical interest in using a combination of either the 5-HT_{2A} receptor antagonist M100907 or the 5-HT_{2A} receptor inverse agonist Pimavanserin combined with the 5-HT_{2C} receptor agonist Lorcaserin. M100907, or Volinanserin, was recently studied in clinical trials for treatment of insomnia and depression (clinicaltrials.gov). Pimavanserin is an FDA-approved drug for the treatment of Parkinson's disease psychosis and is classified as a 5-HT_{2A} receptor inverse agonist (Friedman, 2013; Meltzer et al., 2010). Lorcaserin was previously FDA-approved for treatment of obesity (Gustafson et al., 2013). During a Phase 1 clinical trial, Lorcaserin decreased cocaine craving in cocaine users (Johns et al., 2021). Additionally, studies report that Lorcaserin significantly reduces cannabis self-administration and reduces cannabis craving in humans (Arout et al., 2021), and reduces cannabis, alcohol and nicotine intake in humans (Campbell et al., 2021). Lorcaserin may be effective at treating MUD as well, with a study reporting that Lorcaserin reduces methamphetamine intake in rhesus macaques (Gerak et al., 2016). Future studies should continue examining the serotonergic system in relation to

substance use disorders, and, as previously mentioned, a combination of pharmacotherapies may yield better outcomes and present a more efficacious route for psychostimulant use disorders.

5. Conclusion

In summary, the present results indicate that blocking 5-HT_{2A} receptors with the inverse agonist M100907 does not attenuate expression of methamphetamine-induced CPP, suggesting that blocking 5-HT_{2A} receptors does not decrease contextual-induced drug seeking for methamphetamine. Given that M100907 reduces the reinforcing and rewarding effects of psychostimulants, a broader dose range should be investigated for this experimental design. Additionally, a promising direction of methamphetamine research could consist of combining 5-HT_{2A} antagonists, such as M100907 or Pimavanserin, with 5-HT_{2C} agonists, such as Lorcaserin (Anastasio et al., 2020; Kohut et al., 2014). This approach might result in a more effective MUD pharmacological treatment, maximizing the effects of targeting both receptors to reduce drug seeking while minimizing potential side effects (e.g., locomotor effects). Future research should continue to investigate the link between the serotonergic system and its influence in methamphetamine abuse. Previous studies have reported conflicting results with M100907 decreasing the rewarding and reinforcing effects of stimulants (Nic Dhonnchadha et al., 2009; Fletcher et al., 2012; Madden et al., 2020; 2021), while other studies report no change in drug-related behavior (Bubar & Cunningham, 2008; Banks et al., 2016). Replication and extension of the present results is needed to fully clarify the ability of M100907 to alter methamphetamine abuse-related behaviors.

6. References

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