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Antagonizing serotonin 2A (5-HT2A) receptors attenuates methamphetamine-induced reward and blocks methamphetamine-induced anxiety-like behaviors in adult male rats

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BY

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Antagonizing serotonin 2A ($5\text{-}HT_{2A}$) receptors attenuates methamphetamine-induced reward and blocks methamphetamine-induced anxiety-like behaviors in adult male rats

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ABSTRACT

Methamphetamine (Meth) is a highly addictive and widely abused psychostimulant. Research indicates that the selective $5\text{-}HT_{2A}$ receptor antagonist M100907 attenuates several psychostimulant-induced behaviors, including conditioned place preference (CPP). However, these findings have not yet been extended to Meth. The present study investigated the effects of M100907 on acquisition of Meth-CPP and Meth-induced anxiety.

Adult male rats were tested using an unbiased two-chamber apparatus across eight consecutive days. Prior to Meth administration, rats were pretreated with M100907 and placed into their initially non-preferred chamber. After four Meth conditioning sessions, the effects of M100907 on Meth-induced changes in CPP were assessed. Following CPP testing, rats were screened for anxiety-like behaviors in the elevated plus-maze.

Pretreatment with M100907 attenuated Meth-induced CPP, blocked Meth-induced increases in anxiety-like behavior and attenuated some indices of anxiety in Meth naïve rats. Results suggest that blocking $5\text{-}HT_{2A}$ receptors with M100907 attenuates the rewarding and anxiety-inducing effects of Meth.

Keywords: Methamphetamine, $5\text{-}HT_{2A}$, M100907, Reward, Anxiety

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1. Introduction

Methamphetamine (Meth) is a potent and highly addictive psychostimulant that is one of the most commonly abused drugs globally (Chomchai and Chomchai, 2015). In the United States, abuse of amphetamines in general is rising (Degenhardt, 2010) with Meth abuse reaching epidemic levels (Gonzales et al., 2010). Indeed, as many as 4.7 million people have tried Meth at least once, with nearly a million people meeting criteria for Meth use disorder (Adams et al., 2016). Annual cost estimates of meth abuse are over $23 billion, with the true economic burden ranging between $16.2 to $48.3 billion (Gonzales et al., 2010; Nicosia et al., 2009). Chronic Meth abuse produces numerous negative health consequences including impairments in cognition and memory, symptoms of psychosis, enhanced impulsivity, as well as intense symptoms of withdrawal including drug craving, depression and anxiety (Courtney and Ray., 2014; Glasner-Edwards et al., 2010; Potvin et al., 2018; Rusyniak., 2013). Currently, there are no FDA approved pharmacological treatments for Meth use disorders (Ballester et al., 2016), highlighting the need for research examining the neurobiological mechanisms underlying the euphoric or rewarding effects of Meth, as well as the factors driving continued Meth abuse including symptoms of withdrawal (Härtel-Petri et al., 2017; Hellem, 2016; Marshall and Werb, 2010; Panenka et al., 2013).

The serotonin 2 (5-HT_2) family of receptors are implicated in various neuropsychiatric disorders, including depression (Berg et al., 2008; Celada et al., 2004), anxiety (Weissstaub et al., 2006), Parkinson's disease psychosis, schizophrenia (Abdolmaleky et al., 2004; Sahli and Tarazi, 2018; Talvik-Lofti et al., 2000; Williams et al., 1997) and more recently drug addiction (Bubar and Cunningham, 2008; Cunningham et al., 2012). Consequently, 5-HT_2 receptors have become prime targets for developing novel

pharmacotherapies (Cummings et al., 2018; Meltzer and Roth, 2013; Sahli and Tarazi, 2018). In the addiction field, 5-HT_{2A} receptors are implicated in mediating several psychostimulant-related behaviors including cue-induced reinstatement or extinguished drug-seeking behavior (Fletcher et al., 2002; Nic Dhonnchadha et al., 2009; Pockros et al., 2011), discriminative stimulus effects (Munzar et al., 2002) and hyperlocomotor activity (Steed et al., 2011). A growing body of evidence suggests that the highly selective 5-HT_{2A} receptor antagonist/inverse agonist M100907 (Kehne et al., 1996; Sorensen et al., 1993) may be an effective agent for alleviating addiction-related behaviors. For instance, pretreatment with M100907 decreases cocaine- (Burton et al., 2013; Fletcher et al., 2002; McMahon et al., 2001a; Pockros et al., 2012) and methamphetamine- (Steed et al., 2011) induced hyperlocomotion, cocaine-induced impulsivity (Anastasio et al., 2011; Fletcher et al., 2011) and the discriminative-stimulus effects of cocaine (McMahon and Cunningham, 2001. Interestingly, M100907 does not attenuate cocaine (Fletcher et al., 2002; Nic Donnchadha et al., 2009; Pockros et al., 2011) or nicotine (Fletcher et al., 2012) self-administration in rats, but reduces cue-induced reinstatement for both drugs (Fletcher et al., 2002; Fletcher et al., 2012; Nic Donnchadha et al., 2009). In contrast, a recent report indicates that M100907 attenuates Meth self-administration in primates (Odabas-Geldiay et al., 2019). Collectively, these data suggest that 5-HT_{2A} receptor antagonists may represent a novel target for treating Meth use disorder by interfering with learned associations between stimulant exposure and environmental cues, and/or attenuating Meth reinforcement/reward.

Anxiety is one of the most prevalent psychiatric side-effects of Meth use (Darke et al., 2008; Glasner-Edwards et al., 2010; Zweben et al., 2004) and withdrawal from chronic Meth abuse is associated with severe symptoms of anxiety that can help drive relapse

(Härtel-Petri et al., 2017; Hellem, 2016; Marshall & Werb, 2010). Therefore, an ideal pharmacotherapy would not only reduce the rewarding properties of Meth but would also alleviate withdrawal-induced anxiety. Importantly, in addition to a role in addiction-related behaviors, 5-HT_{2A} receptors represent a novel target for developing anxiolytic drugs (for review see Bourin and Nic Dhonnchadha, 2005). In rodents, chronic Meth exposure produces long-term increases in anxiety-like behaviors during testing in the open field and elevated plus-maze (EPM) paradigms (Hayase et al., 2005; Hrubá et al., 2012; Jang et al., 2013; Loxton & Canales, 2017). Additionally, chronic Meth administration elevates 5-HT_{2A} receptor expression in the medial prefrontal, medial frontal and perirhinal cortices (Chiu et al., 2014; Hámor et al., 2018), regions involved in modulating anxiety (Hannesson et al., 2005; Pati et al., 2018; Pentkowski et al., 2013; Robinson et al., 2016). Notably, the non-selective $5HT_{2A/2C}$ receptor antagonists ritanserin and ketanserin produce anxiolytic-like effects in Meth naïve rats during testing in the EPM (Critchley & Handley, 1987; Motta et al., 1992). Collectively, these data suggest that Meth-induced anxiety-like behavior might be, at least partially, 5-HT_{2A} receptor mediated and that blocking $5HT_{2A}$ receptors could represent a novel target for preventing anxiety-induced relapse.

In the present study we hypothesized that blocking 5-HT_{2A} receptors prior to Meth exposure would attenuate the rewarding effects of Meth and prevent the development of Meth-induced anxiety-like behavior. The role of 5-HT_{2A} receptors in Meth reward was evaluated using the conditioned place preference (CPP) model, which examines classically conditioned associations between the rewarding properties of a drug and the drug context (Bardo & Bevins, 2000; Tzschentke, 2007). As such the drug-taking context acquires conditioned incentive motivational properties (i.e., enhanced incentive salience or value) that

reflect the rewarding properties of the drug (Crombag et al., 2008; Hogarth et al., 2010; Tolliver et al., 2010). These motivational properties persist for weeks after withdrawal, are highly resistant to extinction (de Wit & Stewart, 1981; Mueller et al., 2002), and can promote drug-seeking behavior and relapse (Crombag et al., 2008; O'Brien et al., 1992; Robinson & Berridge, 1993; Stewart, 1983). Thus, use of the CPP paradigm allowed us to evaluate whether M100907 pretreatment reduces Meth-seeking behavior by testing whether it reduces and/or prevents the acquisition of a CPP for the distinct context associated with Meth exposure (Napier et al., 2013; Spanagel, 2017). Following expression testing, rats were screened for anxiety-like behaviors in the EPM. The EPM is by far the most widely used rodent model to examine anxiety-like behavior (for review see Griebel & Holmes, 2013), which generates a conflict in rodents based on their motivation to explore novel environments and the aversive properties of the open arms (Pellow et al., 1985). We report that pretreatment with M100907 reduced acquisition of Meth reward and blocked Meth-induced anxiety-like behavior. Collectively, these findings indicate that 5-HT_{2A} receptor antagonists may represent a novel pharmacological target for treating Meth use disorders.

2. Materials and Methods

2.1. Animals

Subjects were adult male Long-Evans hooded rats (N=68; 90 – 100 days old) born and reared in the Logan Hall breeding colony at the University of New Mexico. Following weaning (21 days), all rats were pair housed with cage mates matched for age from separate litters at 22 °C on a reverse 12-hour dark/light cycle (lights off at 8 am). Harlan 2920 irradiated rodent diet chow and tap water were available *ad libitum* for the duration of the study. All rats were handled daily for one week prior to testing; following weening rats were

handled weekly. All husbandry and procedures adhered to the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and all experimental procedures were reviewed and approved by the University of New Mexico Institutional Animal Care and Use Committee.

2.2. Drugs

M100907 (Axon Medchem LLC, Reston, VA, USA) was dissolved in 0.9% sterile saline containing 3% Tween (vehicle) and Meth hydrochloride (Sigma Aldrich, St. Louis, MO, USA) was dissolved in 0.9% sterile saline. All drugs were administered intraperitoneally (i.p.) at a volume of 1 ml/kg of body weight. The dose of Meth (0 or 1 mg/kg, i.p.) was based on previous research indicating that the 1 mg dose reliably induces CPP in adult rats (Hofford et al., 2014; Xu et al., 2016; Zakharova et al., 2009). The doses of M100907 (0, 0.0025, 0.025 or 0.25 mg/kg, i.p.) were based on previous research indicating that the higher dose attenuated both cocaine- (Pockros et al., 2012) and Meth-induced (Steed et al., 2011) hyperactivity.

2.3. Behavioral Testing

2.3.1. Conditioned Place Preference

The CPP trials were conducted in Plexiglass chambers that contained two equal sized compartments separated by a solid removable partition. The compartments were 25×20×45 cm high and distinguished by wall color, flooring and odor. One compartment contained black walls with bar floors and pine bedding, while the second compartment contained white walls with grid floors and corn cob bedding. During baseline and expression test days (see section 2.3.) the removable solid partitions were replaced with “open” partitions that contained an 8×8 cm opening, allowing rats free access to both compartments.

CPP was conducted across 8 consecutive days in the following order: 1 habituation session, 2 baseline preference assessments, 8 drug conditioning sessions (2/day) and a CPP expression test trial. During the habituation and baseline trials, rats were individually placed into conditioning chambers and allowed to freely explore the apparatus for 15 minutes. During the baseline trials, the total duration of time spent in both the white and black compartments was averaged across both days to determine the rats initial side preference. The compartment in which rats spent less than 50% of their time was designated as the initially "non-preferred side"; the other compartment was designated as the "preferred side". Following baseline assessment, conditioning commenced. Rats were assigned to 1 of 7 groups counterbalanced for initial side preference: vehicle-saline (Veh-Sal), vehicle-Meth (Veh-Meth), low dose M100907-Meth (MLow-Meth), medium dose M100907-saline (MMed-Sal), medium dose M100907-Meth (MMed-Meth), high dose M100907-saline (MHigh-Sal), and high dose M100907-Meth (MHigh-Meth). Each conditioning day consisted of 2 30-minute sessions separated by 4-hours. During one conditioning session, rats received an injection of Meth (1.0 mg/kg/ml, i.p.) and were immediately confined to their initially non-preferred compartment. During the other conditioning session, rats received an injection of saline (1 ml/kg, i.p.) and were immediately confined to their initially preferred compartment. Pairing Meth with the nonpreferred side (referred to as a "biased design") allows for increased sensitivity in detecting "preference shifts". Drug injections were counter-balanced across each conditioning day to ensure that half of the rats received their assigned dose of Meth during the first conditioning session, while the other half received their assigned dose of Meth during the second conditioning session; order of presentation alternated across each conditioning day. M100907 (0.0, 0.0025, 0.025 or 0.25 mg/kg/ml, i.p.)

was administered 15 minutes prior to each conditioning session. Following the fourth and final day of conditioning, the effects of M100907 on acquisition of Meth-induced CPP were assessed by giving rats free access to both conditioning compartments during a 15-minute expression test trial. M100907 and Meth were not administered prior to expression testing. The duration of time spent in both compartments was compared to initial baseline preferences. "Preference shifts" were determined by subtracting each rats' time spent in their initially non-preferred compartment (i.e. baseline) from their time spent in the same compartment during expression testing.

All CPP sessions were monitored and recorded using Lorex infrared surveillance cameras and a Lorex LHV210800 DVR. During baseline and expression trials, the durations of time spent in the white and dark compartments were live-scored by two highly trained overserves blind to group assignments. All testing procedures were conducted in a dimly lit room under red light during the rats' dark cycle. A white noise generator was set at 15 dB to minimize noise disturbances. Between all trials each apparatus was cleaned and wiped down with a 70% ethanol solution to eliminate any residual olfactory cues.

2.3.2. Elevated Plus-Maze

The day following the CPP expression test, the effects of M100907 on Meth-induced anxiety-like behaviors were examined in the EPM. The apparatus consisted of 4 Plexiglass arms (10 wide × 50 cm long) arranged in a plus-shaped configuration, raised 75 cm above the floor. The arms were joined by 10×10 cm center platform. The two "open" arms had no surrounding walls allowing rats to freely observe the surrounding environment, while the two "closed" arms had 40 cm tall walls providing rats protection from the perceived potential for

predation. The front panel of each closed arm was made of clear Plexiglas to allow for observation and recording.

Rats were placed directly on the center platform of the apparatus, facing into one of the two closed arms and were allowed to freely explore the apparatus during a 5-minute test session. Behavioral measures included "avoidance" – the percentage of time spent in the open vs. closed arms; risk assessment behaviors including the number of "head-outs" – extension of the head out of the closed arm, "stretch approach" – movement toward an open arm with a stretched neck and flattened back and "stretch attend" – flattened back and stretched neck attending to the open arms; "head dips" – extension of the head below the edge of an open arm; and "open arm entries" – all four paws moving from a closed- to an open-arm. Note that the three risk assessment behaviors were pooled into a single "risk assessment" measure.

All EPM test trials were recorded using Lorex infrared surveillance cameras and a Lorex LHV210800 DVR. The percentage of time spent in the open vs. closed arms was analyzed using ANY-maze automated tracking software (Stoelting, Wood Dale, IL, USA) and the remaining behavioral measures were scored by a highly trained observer blind to group assignments. All testing procedures were conducted in a dimly lit room under red light during the rats' dark cycle. A white noise generator was set at 15 dB to minimize noise disturbances. Between all test trials the apparatus was cleaned and wiped down with a 70% ethanol solution to eliminate any residual olfactory cues.

2.4. Data Analysis

Meth-induced CPP was operationally defined as a significant increase in the duration of time spent in the initially non-preferred side post-conditioning compared to the pre-

conditioning baseline. Meth-induced CPP was analyzed using a mixed factor ANOVA with Day (baseline vs. expression) as the repeated measure factor, and Meth and M100907 doses as between subjects factors. This operational definition of CPP is the most powerful measure since it involves a within subjects analysis; however, in instances where there were less than three groups, a difference score of time spent in the Meth-paired side post-conditioning minus pre-conditioning (i.e., difference score) was used as the dependent variable to allow for post-hoc comparisons. Separate one-way ANOVAs were used to analyze the effects of M100907 on each dependent measure in the EPM. Least Significant Difference (LSD) post-hoc tests, as well as independent or paired sample t-tests were used to further analyze between and within group differences where appropriate. Cohen's D was computed to provide effect sizes for significant results. All analyses were conducted using SPSS 25, with $\alpha \leq 0.05$ as the threshold for significance. Data are presented as mean ± standard error using GraphPad Prism 7.0; for ease of comparison CPP data are depicted as difference scores. Two rats were excluded from the analysis, with one rat from the Veh-Meth group showing an extreme aversion to Meth and one rat from the Veh-Sal group showing an extreme place preference to saline (greater than 2 standard deviations from the mean in each case). Additionally, due to technical difficulties, EPM data from 12 rats were lost and thus excluded from the analysis.

3. Results

3.1. Methamphetamine Induces a Conditioned Place Preference

Figure 1A displays the preference shifts of saline (Veh-Sal) and Meth (Veh-Met) conditioned rats. The ANOVA revealed a significant Day × Meth group interaction ($F(1,61) = 40.790$, $p = 0.000$). Paired sample t-tests revealed that rats in the Veh-Meth group ($t(9) = -$

8.04, $p = 0.000$, $d = 3.76$) but not the Veh-Sal group ($t(8) = 0.883$, $p = 0.403$, $d = 0.298$) exhibited robust CPP, indicating that the 1 mg/kg dose of Meth was rewarding.

3.2. M100907 Attenuates Methamphetamine-Induced Conditioned Place Preference

Figure 1B displays the effects of M100907 pretreatment on Meth-induced preference shifts. The ANOVA indicated a Day × Meth group × M100907 group interaction ($F(2,61) = 3.09$, $p = 0.05$). Post hoc LSD tests on preference shifts revealed that compared to Meth controls (Veh-Meth), M100907 significantly attenuated preference for the Meth-paired environment (MHigh-Meth: $p = 0.019$; MMed-Meth: $p = 0.035$; MLow-Meth: $p = 0.088$). There were no differences between the three M100907-Meth groups ($p = 0.605$). Collectively, these data indicate that pretreatment with M100907 attenuated Meth-CPP.

3.3. M100907 Does Not Induce a Conditioned Place Preference or Aversion

Figure 1C displays the effects of M100907 on preference shifts in Meth naïve rats. There were no statistically significant difference between groups exposed to M100907 or saline ($F(2,26) = 0.396$, $p = 0.677$), indicating that these M100907 doses do not produce intrinsic reward or aversion.

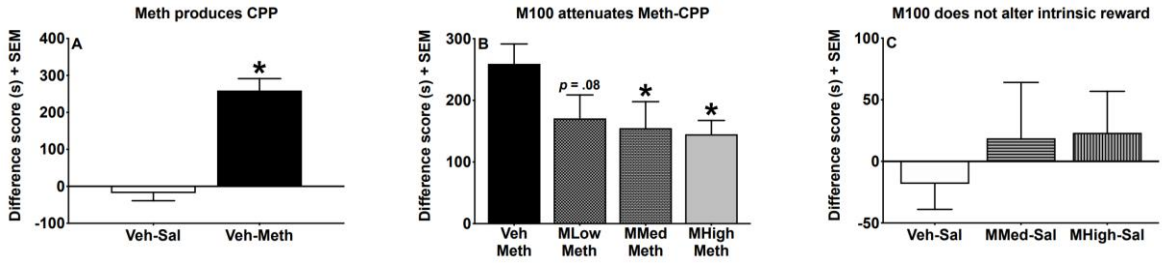

Figure 1: Effects of M100907 (M100) on acquisition of methamphetamine (Meth)-induced conditioned place preference (CPP). There was a main effect of Meth in M100 naïve rats, with Meth (Veh-Meth: n = 10) conditioned rats spending significantly more time in the initially non-preferred compartment compared to saline (Veh-Sal: n = 9) conditioned rats (1A). In Meth conditioned rats, pretreatment with M100 (MMed-Meth: n = 10; MHigh-Meth: n = 11; MLow-Meth: n = 8) attenuated Meth-CPP compared to M100 naïve (Veh-Meth: n = 10) controls (1B). In Meth naïve rats, there were no group differences between control (Veh-Sal: n = 9) and M100 (MMed-Sal: n = 10; MHigh-Sal: n = 10) groups indicating that M100 did not produce intrinsic reward or aversion (1C). Data are expressed as difference scores ± SEM in seconds (s) between the time spent in the initially non-preferred compartment (i.e., baseline) minus test day (i.e., expression). Asterisks (*) represent significant differences between control and experimental conditions ($p < 0.05$ in each case).

3.4. Methamphetamine Increases Anxiety-Like Behavior

Figure 2A displays the percentage of open arm time in saline (Veh-Sal) and Meth (Veh-Meth) conditioned rats. A paired sample t-test indicated that compared to the Veh-Sal controls, rats in the Veh-Meth group spent significantly less time exploring the open arms ($t(15) = 2.467$, $p = 0.026$, $d = 1.20$), indicating that Meth increased anxiety-like behavior. There were no group differences in risk assessment ($p = 0.672$), the number of open arm entries ($p = 0.408$), head dips ($p = 0.094$) or total distance traveled ($p = 0.805$); see table 1 for group means).

3.5. M100907 Blocks Methamphetamine-Induced Anxiety-Like Behavior

Figure 2B displays the effects of M100907 pretreatment on Meth-induced decreases in the duration of time spent exploring the open arms. One-way ANOVA indicated a significant effect of M100907 dose ($F(2,22) = 3.454$, $p = 0.050$). Post hoc LSD tests revealed that compared to Meth controls (Veh-Meth), M100907 significantly increased the percentage of open arm duration (MMed-Meth: $p = 0.025$; MHigh-Meth: $p = 0.052$). The effects of M100907 on risk assessment nearly reached significance ($F(2,22) = 3.287$, $p = 0.056$), with post hoc tests indicating that the MMed group exhibited decreased levels of risk assessment compared to Veh-Meth controls ($p = 0.018$). There were no group differences in number of head dips ($p = 0.853$). open arm entries ($p = 0.772$) or total distance traveled ($p = 0.874$); see table 1 for group means).

3.6. M100907 Attenuates Anxiety-Like Behavior in Methamphetamine Naïve Rats

Figure 2C displays the effects of M100907 on the percentage of open arm time in Meth naïve rats. One-was ANOVA did not indicate a significant effect of M100907 on the percentage of open arm time ($F(2,22) = 1.029$, $p = 0.376$). However, ANOVAs revealed

significant group differences on the number of open arm entries ($F(2,22) = 1.029$, $p = 0.376$) and head dips ($F(2,22) = 6.693$, $p = 0.006$); risk assessment ($F(2,22) = 3.051$, $p = 0.070$) and total distance travelled ($F(2,22) = 3.281$, $p = 0.059$) nearly reached significance; see Table 1 for group means. Post hoc analysis indicated that rats in the MHigh-Sal group entered the open arm more compared to Veh-Sal controls ($p = 0.019$), and that rats in both the MHigh-Sal ($p = 0.032$) and MMed-Sal ($p = 0.002$) groups exhibited more head dips compared to Veh-Sal controls. Collectively, although the main dependent variable of open arm time was not significant, additional behavioral indices suggest that M100907 attenuates anxiety-like behaviors in Meth naïve rats.

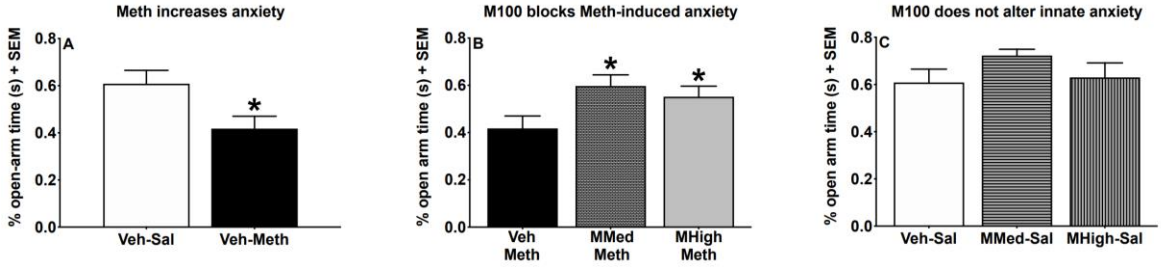

Figure 2: Effects of M100907 (M100) on the methamphetamine (Meth)-induced anxiety-like behavior in the elevated plus-maze (EPM). There was a main effect of Meth in M100 naïve rats, with Meth (Veh-Met: n = 10) exposed rats spending significantly less time in the open arms compared to saline (Veh-Sal: n = 9) exposed rats (2A). In Meth exposed rats, pretreatment with M100 (MMed-Meth: n = 10; MHigh-Meth: n = 11; 2B) blocked Meth-induced anxiety-like behavior compared to M100 naïve (Veh-Meth: n = 10) controls (2B). In Meth naïve rats, there were no group differences between control (Veh-Sal: n = 9) and M100 (MMed-Sal: n = 10; MHigh-Sal: n = 10) groups indicating that M100 did not alter anxiety-like behavior (2C). Data are expressed as the percentage (%) of time spent in seconds (s) in the open arms of the EPM ± SEM. Asterisks (*) represent significant differences between control and experimental conditions ($p < 0.05$ in each case).

	Meth exposed			Meth naive		
Behaviors	Veh-Meth	MMed-Meth	MHigh-Meth	Veh-Sal	MMed-Sal	MHigh-Sal
Open arm entries	13.89 ± 1.49	12.50 ± 1.28	14.2 ± 1.74	12.38 ± 0.86	9.67 ± 1.31	17.44 ± 1.84*
Distance travelled (m)	10.86 ± 0.59	11.20 ± 1.20	10.46 ± 1.07	10.58 ± 0.97	8.99 ± 1.01	12.09 ± 0.58
Risk Assessment	3.56 ± 0.75	1.33 ± 0.33^{+}	2.50 ± 0.43	4.00 ± 0.65	1.33 ± 0.49$^{\&}$	3.78 ± 0.74
Head Dips	10.88 ± 1.49	11.17 ± 2.50	9.60 ± 1.99	7.57 ± 0.95	16.50 ± 1.96*	12.33 ± 2.09*

Table 1: Effects of M100907 on anxiety-like behaviors in methamphetamine (Meth)-exposed rats and Meth-naïve rats during testing in the elevated-plus maze. The asterisk (*) indicates a significant difference compared to the Veh-Sal control group. The appersand ($^{\&}$) indicates a statistical trend compared to the Veh-Sal control group. The plus $^{(+)}$ sign indicates a statistical trend compared to the Veh-Meth control group.

4. Discussion

Results from the present study are the first to indicate that blocking $5HT_{2A}$ receptors with the selective antagonist/inverse agonist M100907 prior to Meth administration attenuates acquisition of Meth-CPP. Specifically, compared to saline conditioned rats (Veh-Sal), Meth conditioned rats (Veh-Meth) exhibited robust CPP (Figure 1A), with M100907 pretreatment attenuating the rewarding effects of Meth (Figure 1B). Importantly, neither dose of M100907 altered place preference in Meth naïve rats, indicating that M100907 does not contain any intrinsic rewarding or aversive properties (Figure 1C). The present results are also the first to report that the $5\text{-}HT_{2A}$ receptor antagonist M100907 can block Meth-induced anxiety-like behaviors. Meth conditioned rats (Veh-Meth) spent significantly less time exploring the open arms of the EPM compared to saline conditioned rats (Veh-Sal; Figure 2A), indicating that Meth increased anxiety-like behaviors. In the Meth-exposed rats, pretreatment with M100907 significantly increased the amount of time exploring the open arms of the EPM (Figure 2B) and significantly reduced risk assessment (Table 1). Interestingly, while open arm durations in Meth naïve groups (Veh-Sal, MMed-Sal, MHigh-

Sal) did not significantly differ (Figure 2C), additional behavioral measures sensitive to standard anxiolytic drugs (e.g., head dips and open arm entries; Carobrez and Bertoglio, 2005; Rodgers et al., 1997) were higher in M100907 groups (Table 1), indicating that M100907 decreases certain indices of anxiety-like behavior. Based on these findings 5-HT_{2A} receptor antagonism may represent a novel mechanism for treating Meth use disorder. Blocking 5-HT_{2A} receptors could reduce Meth withdrawal-induced anxiety and could attenuate the conditioned incentive motivational properties induced by exposure to Meth-paired contexts/cues, thereby reducing drug-seeking behavior and relapse. Importantly, research indicates that like rodents, humans show a CPP for amphetamine (Childs and de Wit, 2009) and a conditioned preference for a Meth-associated contextual cue (Mayo et al., 2013), offering translational validity for the present results.

Mechanistically, the present behavioral effects of M100907 likely involve a reduction in stimulant-induced mesocorticolimbic dopaminergic systems. Studies using positron emission tomography and in-situ hybridization histochemistry indicate that 5-HT_{2A} receptors are expressed in key addiction-related circuits including robust expression on pyramidal and GABAergic neurons throughout the human prefrontal cortex (PFC) (Burnet et al., 1995; Hall et al., 2000), with moderate expression on pyramidal neurons in fields CA1 of the hippocampus (Pasqualetti et al., 1996). Similar techniques in rodents indicate that 5-HT_{2A} receptors are also richly expressed on pyramidal neurons in the PFC (Pompeiano et al., 1994) and hippocampus, and on dopaminergic neurons in the ventral tegmental area (VTA) Doherty & Pickel, 2000). Importantly, chronic Meth exposure in rodents increases 5-HT_{2A} receptor expression throughout the PFC (Chiu et al., 2014; Hámor et al., 2018), suggesting that compulsive drug use may involve enhanced frontal cortical 5-HT_{2A} receptor signaling.

Tract tracing studies in rats indicate that 5-HT_{2A} receptor expressing pyramidal cells in the PFC project to dopaminergic cells in the VTA (Vázquez-Borsetti et al., 2009) and GABAergic cells in the nucleus accumbens (NAc; for review see (Mengod et al., 2015)), suggesting that activation of 5-HT_{2A} receptors in the PFC and/or VTA could enhance dopaminergic signaling that is critically involved in psychostimulant addiction. In support of this hypothesis, systemic and localized (PFC) injections of 5-HT_{2A} receptor agonists enhance dopaminergic firing in the VTA, as well as dopamine release in the VTA and mPFC, effects that are reversed by M100907 (Bortolozzi et al., 2005). Additionally, systemic administration M100907 reduces amphetamine-induced dopamine release in VTA terminals including the NAc and PFC (Pehek et al., 2001; Porras et al., 2002). Thus, blocking 5-HT_{2A} receptors in the present study could decrease activation of excitatory PFC projections to the VTA or decrease VTA neuronal activity directly, thereby attenuating dopamine release in VTA terminal regions implicated in modulating drug abuse (Koob & Volkow, 2016; Walker & Nestler, 2018)- and anxiety (Gross and Canteras, 2012; Perusini and Fanselow, 2015)-related behaviors including the PFC, NAc, caudate-putamen (CPu), hippocampus and amygdala. Notably, M100907 attenuates cocaine-induced Fos protein expression in the NAc shell and CPu (Pockros et al., 2012), and reduces amphetamine-induced dopamine release in the NAc and PFC (Pehek et al., 2001; Porras et al., 2002). Collectively, these reports indicate that the activity of VTA dopamine neurons are under the excitatory control of 5-HT_{2A} receptors in the mPFC and VTA, and that 5-HT_{2A} receptor antagonism in these regions likely underlie the present behavioral results.

The present findings partially help resolve the conflicting literature examining the influence of 5-HT_{2A} receptor activity on the rewarding and reinforcing effects of

psychostimulants. Research has shown that peripheral injections (Fletcher et al., 2002; Nic Dhonnchadha et al. 2009) and ventromedial prefrontal cortex infusions (Pockros et al., 2011) of M100907 fail to attenuate cocaine or nicotine (Fletcher et al., 2012) self-administration rates, but successfully block cue-induced reinstatement of drug seeking behaviors in rats (Fletcher et al., 2002, 2012; Nic Dhonnchadha et al. 2009; Pockros et al., 2011). In addition, a study in non-human primates found that Pimavanserin, a 5-HT_{2A} receptor antagonist/inverse agonist, fails to attenuate Meth reinforcement (Banks, 2016); however, these findings are not congruent with a recent study that found that M100907 attenuates Meth self-administration (Odabas-Geldiay et al., 2019). Similarly, the present results indicate that M100907 attenuates acquisition of Meth-induced reward. Collectively, these data suggest that for psychostimulants in general, blocking 5-HT_{2A} receptors reduces the conditioned incentive motivational properties (i.e., enhanced incentive salience or value) of drug associated cues/contexts that can promote drug-seeking behavior and relapse (Crombag et al., 2008; O'Brien et al., 1992; Robinson & Berridge, 1993; Stewart, 1983). For Meth specifically, blocking 5-HT_{2A} receptors with M100907, but not Pimavanserin, attenuates Meth reinforcement/reward. These drug differences may result from M100907 displaying a higher 5-HT_{2A}/5-HT_{2C} receptor selectivity ratio compared to Pimavanserin, as well as being slightly more potent (Vanover et al., 2006). Higher selectivity for 5-HT_{2A} receptors is critical because blocking 5-HT_{2C} receptors can produce the opposite behavioral effect. Taken together, these reports suggest that M100907 may represent a novel pharmacological target for preventing psychostimulant relapse by preventing cue and contextual-induced incentive motivation (i.e., craving), as well as reduce Meth intake if relapse occurs.

Several factors suggest that the present effects of M100907 on Meth-induced reward and Meth-induced anxiety-like behavior were 5-HT_{2A} receptor mediated. First, the effects of M100907 in both tests were dose dependent. While these conclusions are statistically accurate, the doses of M100907 that failed to alter Meth-induced reward or anxiety-like behavior generally approached significance. However, these findings are consistent with prior literature indicating that the low doses of M100907 used in the present report alter psychostimulant-induced behaviors. For instance, using the self-administration paradigm in rats Nic Dhonnchadha and colleagues (2009) reported that a wide range of M100907 doses (0.001 to 0.8 mg/kg, i.p.) significantly reduce cue-induced reinstatement of extinguished cocaine-seeking behavior. Similarly, Pockros and colleagues (2012) reported that M100907 decreases cocaine-induced hyperactivity at low doses (0.05 and 0.1 mg/kg). Lastly, M100907 is a highly selective and potent 5-HT_{2A} receptor antagonist with > 100-fold selectivity for 5-HT_{2A} receptors over any other receptor subtype (Kehne et al., 1996; Sorensen et al., 1993). Collectively, these previous reports along with the present data indicate that M100907 is a potent 5-HT_{2A} receptor antagonist capable of altering stimulant-induced behaviors at low doses.

In the present study we report that anxiety-like behaviors are induced by repeated Meth administration and that this anxiogenic-like effect was completely blocked by M100907 pretreatment. However, when administered in Meth naïve rats M100907 failed to alter the percentage of time exploring the open arms, the main indices of anxiety-like behavior. However, M100907 increased additional indices of anxiety-like behavior (e.g., head dips and open arm entries) similar to the effects of standard anxiolytic drugs (Carobrez and Bertoglio, 2005). Thus, our findings are consistent with the existing literature reporting

that Meth increases anxiety-like behaviors in rats (Hayase et al., 2005; Hrubá et al., 2012; Jang et al., 2013; Loxton & Canales, 2017), however, are results are only partially consistent with literature examining 5-HT_{2A} receptor effects in Meth naïve rodents. For example, the non-selective 5-$HT_{2A/2C}$ receptor antagonists ritanserin and ketanserin produce anxiolytic-like effects in rats (Critchley & Handley, 1987; Motta et al., 1992), but M100907 fails to attenuate anxiety-like behavior in mice (Griebel et al., 1997). The different outcomes between the present results and the null effects in mice may be due to species differences, a prior history of saline conditioning and/or different M100907 doses. In the present study, the anxiolytic-like effects of M100907 were more robust in the Meth exposed group. These differences may be due to the acute and long-term neurobiological alterations produced by chronic Meth exposure. Meth elevates extracellular levels of 5-HT (Berger et al., 1992; Kuczenski et al., 1995) and upregulates 5-HT_{2A} receptor expression in the medial prefrontal, medial frontal and perirhinal cortices (Chiu et al., 2014; Hámor et al., 2018), regions implicated in the modulation of anxiety (Hannesson et al., 2005; Pati et al., 2018; Pentkowski et al., 2013; Robinson et al., 2016). Thus, M100907 may block Meth-induced amplified 5-HT signaling at $5HT_{2A}$ receptors in Meth-exposed rats. Future research examining 5-HT_{2A} receptors in these regions is needed to confirm this hypothesis.

5. Conclusions

In summary, the present results indicate that blocking $5HT_{2A}$ receptors with the selective antagonist/inverse agonist M100907 attenuates the rewarding effects of Meth and blocks Meth-induced anxiety-like behaviors. Given Meth's rewarding and anxiogenic properties, targeting $5HT_{2A}$ receptors with M100907 may represent a novel pharmacological approach for treating Meth addiction. M100907 may attenuate drug-seeking behaviors by

inhibiting Meth-induced reward and/or the incentive motivation properties elicited by Meth-paired cues/contexts, as well as reduce potential anxiety driven relapse by blocking Meth's anxiogenic effects. Given the dense populations of $5HT_{2A}$ receptors in the PFC and VTA (Doherty & Pickel, 2000; Pompeiano et al., 1994), future research should characterize the regional effects of $5HT_{2A}$ receptor antagonism on Meth reward, focusing on its role within the mesocorticolimbic dopaminergic system. While the present study analyzed the effects of M100907 on Meth-induced behaviors in adult male rats, the potential effects in females as well as in adolescent rats needs to be determined. While we can hypothesize that M100907 may similarly affect Meth-induced behaviors in these groups, the magnitude of the effect and range of effective doses may differ. Additionally, future research should examine how M100907 influences Meth reinforcement in rats. While self-administration studies have addressed this in non-human primate models (Banks, 2016; Odabas-Geldiay et al., 2019), the outcomes have been mixed. Lastly, future research should examine the effects of M100907 on expression and extinction of Meth CPP. M100907's ability to block cue-induced reinstatement (Fletcher et al., 2002; Nic Dhonnchadha et al. 2009; Pockros et al., 2011) may, in part, be due to interference with learned associations between psychostimulant exposure and environmental cues, or reduced incentive motivation elicited by cue exposure. If M100907 interferes with the incentive motivational properties associated with the conditioned context, M100907 pretreatment before expression testing or extinction trials should reduce preference for the Meth-paired compartments.

6. References

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