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Assessment of Dopaminergic and Serotonergic Receptor Antagonists in Male Rats Trained to Discriminate 4-Methylmethcathinone (Mephedrone)

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ASSESSMENT OF DOPAMINERGIC AND SEROTONERGIC RECEPTOR ANTAGONISTS
IN MALE RATS TRAINED TO DISCRIMINATE 4-METHYLMETHCATHINONE
(MEPHEDRONE)

by

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ASSESSMENT OF DOPAMINERGIC AND SEROTONERGIC RECEPTOR ANTAGONISTS IN MALE RATS TRAINED TO DISCRIMINATE 4-METHYLMETHCATHINONE (MEPHEDRONE)

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Preclinical drug discrimination studies of the synthetic cathinone, 4-methylmethcathinone (mephedrone) have demonstrated its effects are comparable to those of other popular psychostimulant drugs. Few studies have directly examined the contribution of specific neurotransmitter receptors to mephedrone's discriminative stimulus effects. The present study investigated the role of dopamine and serotonin receptors in these effects. Eight adult male Sprague-Dawley rats were trained to discriminate 3.0 mg/kg mephedrone from saline. After dose-response curves were determined with mephedrone (0.375-3.0 mg/kg), a series of stimulus antagonism tests were conducted with dopamine antagonists (Sch 23390, haloperidol) and serotonin antagonists (WAY 100,635, MDL 100,907, pirenperone) administered as a pretreatment with each mephedrone dose. Attenuation of mephedrone discrimination by Sch 23390 and haloperidol implicates the involvement of both D_1 and D_2 dopamine receptors in these effects. Partial attenuation of discrimination by MDL 100,907 and pirenperone, but not WAY 100,635, indicates $5HT_2$ receptors also contribute to these effects, while $5HT_{1A}$ receptors do not. The absence of full stimulus antagonism with these compounds indicate mephedrone's discriminative stimulus effects may be mediated by a combination of monoamine receptors or other neurotransmitter actions yet to be evaluated. These results contribute to a growing body of literature regarding the interoceptive stimulus effects of mephedrone and serve to inform clinical science regarding the neurochemical mechanisms involved with abuse risks of this substance.

ACKNOWLEDGEMENTS

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INTRODUCTION

Synthetic cathinones are common constituents of the psychoactive 'bath salts' that emerged on the clandestine drug market in the United States and Europe in the early 2000's as an alternative to traditional psychostimulant drugs (Goodnough & Zezima, 2011; Winstock & Ramsey, 2010). These substances are structurally similar to the psychoactive ingredient in *Catha edulis* (Khat), a plant widely consumed primarily in parts of Africa and the southern Arabian peninsula (Sheikh et al., 2014). Because of its association with a myriad of negative health outcomes, from dependence to increased risk of heart attack and stroke, khat is listed as a prohibited substance by the United Nations, as well as outlawed in many countries, including the United States, Canada, and the United Kingdom (Sheikh et al., 2014).

While synthetic cathinones share a similar molecular structure to cathinone, synthetic cathinones such as mephedrone (4-methylmethcathinone, 4-MMC), and 3-4 methylenedioxypyrovalerone (MDPV) are significantly more potent (Gregg et al., 2015). Moreover, mephedrone and MDPV are increasingly found in drugs marketed as 'ecstasy' or 'molly' to unwitting users (Gregg et al., 2015). Medical professionals noted a disturbing trend of severe adverse effects linked to mephedrone, and as a result, in 2014 the United States Drug Enforcement Administration permanently added this substance along with several other synthetic cathinones to the Schedule I list of controlled substances (Drug Enforcement Administration, 2014b; Gibbons & Zloh, 2010; Kasick et al., 2012; Rosenbaum et al., 2012).

Despite attempts to curtail their recreational use through legal restrictions and education, use of synthetic cathinones remains a public health concern (German et al., 2014). The Drug Enforcement Administration's (DEA) National Forensic Laboratories Information System

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(NFLIS) special report on synthetic cannabinoids and synthetic cathinones indicates synthetic cathinone use continues to increase; reports of use nearly doubled from 29,648 in the 2010-2013 reporting period to 51,824 in the 2013-2015 reporting period (Drug Enforcement Administration, 2011, 2014a, 2016). According to the DEA, mephedrone was the most prevalent synthetic cathinone detected in samples seized by law enforcement and submitted to federal, state, and local laboratories participating in the National Forensic Laboratories Information System (NFLIS) in the United States from 2010-2013, and remains among the 20 most commonly reported synthetic cathinones found in samples according to the most recent report (Drug Enforcement Administration, 2014a, 2016).

Scientific research on the psychopharmacology of synthetic cathinones has expanded considerably in recent years, no doubt due to their continued prevalence as recreational drugs of abuse. Several published preclinical studies indicate that the common constituents found in bath salts are comparable in abuse liability and pharmacology to other abused stimulants, such as MDMA, cocaine, or amphetamines (Baumann et al., 2012; Berquist et al., 2017; Cameron et al., 2013; Harvey et al., 2017). Furthermore, the chemical structure of mephedrone resembles prototypical phenylethylamine derivatives, such as MDMA and methamphetamine, and therefore may serve as a useful model for predicting the effects of emerging synthetic cathinones that share a similar structure (Berquist et al., 2017). Among these similar second generation synthetic cathinones, 4-methyl-N-ethylcathinone (4-MEC) and 4′- methyl-α-pyrrolidinopropiophenone (4-MePPP) can already be found alongside mephedrone in the 20 most commonly identified synthetic cathinones in samples submitted to NFLIS laboratories (Drug Enforcement Administration, 2014a).

Neurochemical studies indicate mephedrone is a nonselective substrate for release at dopamine, serotonin, and norepinephrine transporters (DAT, SERT, & NET respectively), similar to the actions of MDMA (Baumann et al., 2012). When administered intravenously, mephedrone increases extracellular serotonin (5-HT), dopamine (DA), and norepinephrine (NE), with a greater effect on 5-HT release (Baumann et al., 2012; Kehr et al., 2011). Additionally, mephedrone is a 5-HT and DA transport inhibitor, with higher affinity for DAT than SERT (Hadlock et al., 2011; Martínez-Clemente et al., 2012). Similar to more traditional psychomotor stimulants, mephedrone produces spontaneous increases in locomotor activity, produces conditioned place preference in rats and mice equal to or greater than that produced by amphetamine at doses as low as 5 mg/kg, and it is readily intravenously self-administered by both male and female rats, consistent with its high abuse liability (Creehan et al., 2015; Huang et al., 2012; Karlsson et al., 2014; Lisek et al., 2012; Motbey et al., 2013; Nguyen et al., 2016).

Drug discrimination has long been regarded a "gold standard" paradigm for predicting abuse-related behavioral effects of psychoactive drugs. Several meta-analyses indicate this paradigm is among the best predictors of scheduling risk, particularly when drugs with known abuse potential are tested for substitution with subjects trained to discriminate the novel compound (Horton et al., 2013; Huskinson et al., 2015). This well-established *in vivo* preclinical behavioral paradigm has also been demonstrated to have exceptional predictive utility for discerning neurochemical mechanisms of drug action (Glennon & Young, 2011). At the time this study was initiated, seven published studies evaluated the discriminative stimulus effects of mephedrone (4-MMC) in rodents (Berquist et al., 2017; DeLarge et al., 2017; Erwin et al., 2019; Gatch et al., 2013; Harvey & Baker, 2016; Saber et al., 2019; Varner et al., 2013). Five of these studies utilized mephedrone as the training stimulus. Collectively, these studies characterized the

interoceptive stimulus effects of mephedrone through tests of stimulus substitution, potentiation, and antagonism. These findings are summarized in Table 1 and Table 2.

Table 1. *Preclinical Findings with Mephedrone: Drug Discrimination Substitution Tests*

Primary Mechanism	Classification	Drug	Substitution
Dopaminergic (DA)	Psychostimulant	Amphetamine	Yes*
		Methamphetamine	Yes*
		Cocaine	Yes*
Mixed DA/ Serotonergic	Empathogen-entactogen	MDMA	Yes
		MDA	Yes
Serotonergic	Hallucinogen	LSD	Partial
	Serotonin Releaser	Fenfluramine	Partial
Opioid receptors	Opioid	Morphine	Partial
		Heroin	Partial
NMDA receptor antagonist	Dissociative anesthetic	Ketamine	Partial
		Phencyclidine	Partial
Cannabinoid	Cannabinoid	delta-9-THC	Partial

Table summarizing the substitution tests from published drug discrimination studies using rats trained to discriminate mephedrone from saline (Berquist et al., 2017; DeLarge et al., 2017; Erwin et al., 2019; Saber et al., 2019; Varner et al., 2013). * indicates substitution was dependent on the mephedrone training dose.

Table 2. *Preclinical Findings with Mephedrone: Drug Discrimination Antagonism and Transport Inhibitor Tests*

Pre-Treatment	Action	Block (Full/None/Partial)	Citation
SCH23390	D_1 Antagonist	Full	Saber et al., 2019
Sulpiride	D_2 Antagonist	Partial	Saber et al., 2019
Haloperidol		None	Varner et al., 2013
SB242084	$5\text{-}HT_{2c}$ Antagonist	None	Saber et al., 2019
Ketanserin	$5\text{-}HT_{2a/2c}$ Antagonist	None	Saber et al., 2019
Ritanserin		None	Erwin et al., 2019
Rimcazole	Sigma Antagonist	Potentiation	DeLarge et al., 2017
Desipramine	NET Inhibitor	Potentiation	DeLarge et al., 2017
Fluoxetine	SERT Inhibitor	Full to Potentiation	DeLarge et al., 2017

Table summarizing the antagonism and transport inhibitor tests from published drug discrimination studies using rats trained to discriminate mephedrone from saline (DeLarge et al., 2017; Erwin et al., 2019; Saber et al., 2019; Varner et al., 2013).

Of the myriad of drugs tested for substitution in rodents trained to discriminate mephedrone, substances with predominantly dopaminergic actions (e.g., amphetamine, methamphetamine) or a mixture of serotonergic and dopaminergic actions (MDMA, MDA) consistently substituted for mephedrone, while drugs with predominantly serotonergic actions (e.g., LSD, fenfluramine) produced only partial substitution (Berquist et al., 2017; DeLarge et al., 2017; Erwin et al., 2019; Saber et al., 2019; Varner et al., 2013). Opioids (morphine, heroin), dissociative anesthetics (ketamine, phencyclidine) and delta-9-THC also produced only partial substitution for mephedrone (Berquist et al., 2017; DeLarge et al., 2017; Erwin et al., 2019; Saber et al., 2019; Varner et al., 2013). Of particular interest, the extent of substitution with psychostimulants (cocaine, (+)-methamphetamine, d-amphetamine) is dependent on the mephedrone training dose, with full substitution observed for low training doses and minimal or partial substitution for high training doses (Berquist et al., 2017; Saber et al., 2019) The sigma receptor antagonist rimcazole and the norepinephrine transport (NET) inhibitor desipramine potentiated the effects of mephedrone, whereas the serotonin transport (SERT) inhibitor, fluoxetine blocked mephedrone discrimination at low doses and potentiated its effects at high doses (DeLarge et al., 2017).

In drug discrimination assessments with antagonists, the D_1 antagonist SCH23390 produced marked decrease in response rates, but blocked the discriminative stimulus effects of mephedrone in the subjects that responded, while the D_2 agonist sulpiride produced partial antagonism in rats that were trained on a low (0.5mg/kg) dose of mephedrone (Saber et al., 2019). Haloperidol, a D_2 antagonist, SB242084, a 5-HT_{2c} antagonist, and 5-$HT_{2a/2c}$ antagonists ketanserin and ritanserin all failed to block the discrimination of mephedrone (Erwin et al., 2019; Saber et al., 2019; Varner et al., 2013). Considered together, these studies indicate that the

discriminative stimulus effects of mephedrone may be partially mediated by both dopaminergic and serotonergic receptor activities.

The aim of the present study was to replicate and expand on previous investigations of mephedrone stimulus antagonism. Two dopamine antagonists (Sch 23390, haloperidol) and three serotonin antagonists (MDL 100, 907, pirenperone, WAY 100,635) were each assessed in rats trained to discriminate 3 mg/kg mephedrone.

METHODS

Subjects

Eight adult male Sprague-Dawley rats (380g-450g) served as subjects. Rats were individually housed in polycarbonate cages with corncob bedding (Teklad, #7097, Envigo, Madison, Wisconsin, USA). The animal facilities were maintained at 22C +/- 2, and humidity 50% +/- 5, with a 12:12 light:dark cycle (lights on at 6/7 depending on daylight savings time). Subjects were maintained at 90% of their average free feed weights and had *ad libitum* access to water. All procedures were reviewed and approved by the Western Michigan University Institutional Animal Care and Use Committee and were in accordance with the guidelines of the Guide for the Care and Use of Laboratory Animals (National Research Council of the National Academies, 2011) and EU Directive 2010/63/EU.

Apparatus

All training and test sessions were conducted utilizing eight sound-attenuated operant conditioning chambers (ENV-001, Med Associates Inc., St. Albans, Vermont, USA), and controlled using Med-PC software (version IV, Med Associates Inc.). Each chamber was equipped with a 28 Volt house light, a ventilation fan, three retractable levers approximately two inches from the floor, and a single stimulus light directly above each the right and left levers. Reinforcement during training sessions consisted of 45 mg Dustless Precision Pellets®

(Product# F0021, Bio-Serv Inc., Flemington, New Jersey, USA) dispensed from a pellet dispenser directly above the center lever. Neither of the stimulus lights were active during training or test sessions.

Drugs

(±)-Mephedrone-hydrochloride (4-MMC) was provided by the National Institute on Drug Abuse Drug Control Supply Program (Bethesda, MD, USA). (R)-(+)-a-(2,3-Dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol (MDL 100,907) and N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide trihydrochloride (WAY 100,635) were provided by the National Institutes of Mental Health Chemical Synthesis and Drug Supply Program. R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH 23390), 3-[2-[4-[(4-fluorophenyl)-oxomethyl]-1-piperidinyl]ethyl]-2-methyl-4-pyrido[1,2-a]pyrimidinone (pirenperone) and haloperidol were purchased from Sigma Chemical Company, Inc. (St. Louis, MO, USA). Haloperidol was first dissolved in a few drops of 0.1 M HCl and diluted in sterile water. Pirenperone was dissolved in 30% 2-hydroxypropyl-beta-cyclodextrin at a concentration of 1 mg/ml and diluted with sterile water. MDL 100,907 was dissolved with a few drops of glacial acetic acid and diluted with sterile water. All other drugs were dissolved in bacteriostatic 0.9% sodium chloride. Drug doses were calculated based on the weights of solid compound and were administered via intraperitoneal (ip) injections at a constant volume of 1 ml/kg body weight. Mephedrone was administered 15 minutes prior to behavioral testing. SCH 23390 was administered 15 minutes before the mephedrone, 30 minutes prior to testing. All other antagonists were administered 45 minutes prior to mephedrone, 60 minutes before behavioral testing.

Procedures

Preliminary Training: Training began with a single 60-minute session, during which no levers were extended, and pellets were delivered on a 60 second fixed time interval, in order to acquaint subjects with the location and sounds of the pellet dispenser. Additionally, after this first session rats were given a supplement of approximately ten precision dustless pellets in their home cages to decrease their novelty and ensure the rats would consume them in subsequent sessions. All following training sessions were 20 minutes in duration and were conducted 5-6 days a week. Preliminary lever training began with only the center lever present, whereby reinforcement was delivered on a fixed ratio 1 (FR 1) schedule that was gradually incremented to a FR 20 schedule across 10-15 daily sessions.

Errorless Training: When responding was stable on an FR 20 schedule, errorless training commenced. During this phase, rats received an intraperitoneal injection of either saline (S) or 3.0 mg/kg mephedrone (D) 15 minutes prior to the start of each session. Rats again were placed on a FR1 schedule that was incremented across four to nine sessions to FR 20. Subjects 1-4 received reinforcement for completing the FR requirement on the right lever following drug injections and the left lever following saline vehicle (V) injections. Conditions were reversed for subjects 5-8.

Discrimination Training: Once reliably responding at an FR20 in the errorless phase, discrimination training commenced. Both errorless training and discrimination training were conducted using the following 12-day pattern: VVDDVDVVDDVD. During discrimination training, both the left and right lever were extended. Completion of the FR on the injection-appropriate lever was reinforced and responses on the other lever reset the response requirement. Criteria for discrimination was set at 80% correct lever responding for eight out of ten

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consecutive training sessions. Once the aforementioned discrimination criteria were met, subjects commenced testing.

Testing Phase: Substitution tests were initially conducted with a range of mephedrone doses (0.375, 0.75, 1.5, 3 mg/kg) and saline to generate a dose response curve. Subsequently, antagonist tests were conducted with a single dose of each antagonist (indicated below) with each of the aforementioned mephedrone doses. Test sessions were conducted in a similar manner to training sessions with the exception that no reinforcers were delivered, and sessions terminated upon completion of the first FR on either lever, or after 20 minutes, whichever came first. Between tests, subjects were required to meet criteria for stimulus control on at least one V and D training session. Antagonists were injected 30 minutes (0.03 mg/kg Sch 23390) or 60 minutes (0.25 mg/kg haloperidol, 0.32 mg/kg pirenperone, 1.6 mg/kg WAY 100,635, 0.1 mg/kg MDL 100,907) prior to each test session followed by one of the aforementioned mephedrone doses 15 min before each test session.

Data Analysis

Sessions to criteria was determined from the first session where both levers were available. Percent drug lever selection was determined by dividing the number of responses emitted on the mephedrone-paired lever by the total number of responses emitted on both levers and multiplying by 100. Response rates were expressed as the number of responses per second during the test session. Subjects that failed to complete 10 total lever presses within the 20-min test session were not considered in the calculation of percent mephedrone-lever selection however were included in the statistical analysis of response rates. Mean percent of drug lever responding and mean response rate for each dose and combination were determined and plotted for visual analysis.

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During initial mephedrone testing to establish the dose-response curve, full substitution was defined as $\geq 80\%$ mephedrone-lever selection, partial substitution was defined as between 20%-79% mephedrone-lever responding, and no substitution was defined as less than 20% mephedrone-lever responding. For antagonism testing antagonism was defined as $\geq 80\%$ mephedrone-lever selection, less than 20% mephedrone lever responding was considered full antagonism, and partial attenuation was considered anywhere between 20%-79% drug lever responding. For each antagonist assessed, the response rate was statistically analyzed with a two-way repeated-measures analysis of variance (RM ANOVA) with antagonist pre-treatment and mephedrone dose as the two independent variables. Statistical significance was determined at alpha of $p < 0.05$. A Holm-Sidak multiple comparison test was conducted following the RM ANOVA. All statistical and graphical analyses were performed using the GraphPad Prism version 7 software (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

All eight rats met the discrimination criteria within an average of 29.8 ($\pm$ 5.9) training sessions (Range:18-62). Mephedrone produced dose-dependent increase in drug-lever responses with full substitution at 1.5 mg/kg and 3.0mg/kg mephedrone. Response rate was reduced by 3.0 mg/kg mephedrone compared to saline and lower mephedrone doses, but differences were not statistically significant.

Dose response curves for mephedrone alone and with each antagonist pre-treatment are displayed in figure 1. The D_1 receptor antagonist, Sch 23390 (0.03 mg/kg) attenuated discrimination of mephedrone at 0.375mg/kg, 0.75mg/kg, and 1.5mg/kg, but failed to block discrimination of the training dose. This antagonist also produced a significant decrease in response rate. A two way RM ANOVA on response rate indicated a statistically significant effect

of pre-treatment with Sch 23390 ($F_{(1,4)}=23.29$, p=0.01) but no significant effect of mephedrone

dose. The D_2 receptor antagonist, haloperidol (0.25 mg/kg) partially attenuated mephedrone

discrimination at 1.5 and 3.0 mg/kg. Haloperidol appeared to produce a decrease in response

rate, and completely eliminated responding in two animals, however a two way RM ANOVA

determined that there was no statistically significant effect of pre-treatment $F_{(1,5)}=6.06$,
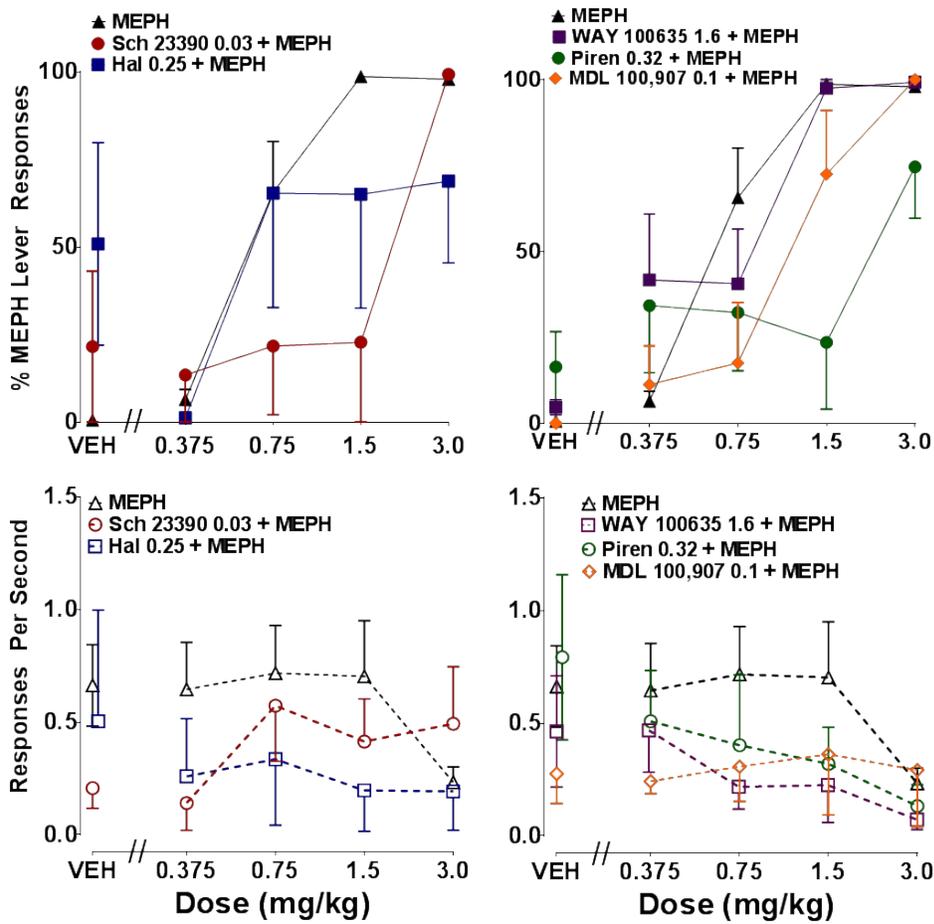
p=0.06), nor was there an effect of mephedrone dose.

Figure 1. **Pretreatment with Dopamine and Serotonin Antagonists in Male Rats**
Both Sch 23390 and haloperidol attenuated discrimination of low MEPH doses, but disrupted responding in at least half the animals, precluding statistical analysis. The 5-HT_2 antagonists, pirenperone and MDL produced a rightward shift in the MEPH dose response curve, whereas the 5-HT_{1A} antagonist, WAY 100,635 did not alter MEPH discrimination.
(4-MMC, N=8; Sch 23390, N=6-7; Hal, N=3-4; Piren, N=5-7; WAY, N=7-8; MDL, N=5)

The 5-HT_{1A} antagonist, WAY 100635 (1.6 mg/kg) failed to block discrimination of

mephedrone at any dose. Of the seven rats to complete testing, WAY 100635 completely

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blocked discrimination in one, partially blocked in another, and failed to block in the remaining five animals. Of interest, WAY 100,635 appeared to potentiate low doses of mephedrone; when administered with 0.375 mg/kg mephedrone, drug lever responding increased (X=41.9%) compared to the 0.375 dose of mephedrone alone (X=21.6%). When tested with 0.375 mg/kg mephedrone alone, only one animal responded exclusively on the drug lever. In comparison, pre-treatment with WAY 100,635 prior to 0.375mg/kg mephedrone produced full substitution in three animals (96.6%, 100%, 92%).

The 5-HT$_{2A}$ antagonist, MDL 100,907 (0.1 mg/kg) and the less selective 5-HT$_2$ antagonist, pirenperone (0.32mg/kg) produced partial attenuation of mephedrone discrimination and produced a rightward shift in the mephedrone dose response curve. Although pre-treatment with these antagonists suppressed response rate compared to the effects of mephedrone alone, these effects were not statistically significant. A two way RM ANOVA on response rate following antagonism pretreatments revealed no main effect of WAY100,635 pretreatment [$F(1,7)$=4.427, p=0.07]), MDL100,907 pretreatment [$F(1,3)$=4.546, p>0.1], or pirenperone pretreatment [$F(1,4)$=0.5972, p=0.4828], as well as no significant effect of mephedrone dose or dose by pre-treatment interaction.

DISCUSSION

The present study examined the contribution of serotonergic and dopaminergic mechanisms to the discriminative stimulus effects of mephedrone through antagonism testing in rats trained to discriminate this synthetic cathinone from saline. Although stimulus control was established in all eight rats, the number of sessions required to meet criteria was variable. Five of the subjects achieved criteria for stimulus control in 18-19 sessions, while the remaining three required 40, 44, or 62 sessions to meet these criteria. This disparity may indicate differing levels

of sensitivity to mephedrone, though other methodological variables related to training cannot be ruled out. Nonetheless, the number of training sessions required to establish stimulus control in the current study were comparable to that reported in previous mephedrone drug discrimination studies despite a number of methodological differences including rodent strain and training methods (DeLarge et al., 2017; Erwin et al., 2019; Saber et al., 2019; Varner et al., 2013).

A limited number of published studies have evaluated the effects of dopamine antagonists on mephedrone discrimination. Saber et al. (2019) reported partial attenuation of mephedrone discrimination with the D_2 antagonist, sulpiride. Consistent with those findings, the current study found partial attenuation of mephedrone discrimination by Sch 23390 and haloperidol. These findings implicate the involvement of D_1 and D_2 dopamine receptor activities in the interoceptive effects of mephedrone. However, the lack of full stimulus antagonism suggests other receptor mechanisms may also contribute to these effects. Whereas these DA antagonists also substantially suppressed responding, their effects on mephedrone discrimination should be interpreted with caution. The 5-HT_2 antagonists MDL 100,907 and pirenperone also partially attenuated mephedrone discrimination with less overall disruption of response rate. Failure of WAY 100, 635 to alter mephedrone discrimination suggest 5-HT_{1A} receptors do not contribute to these effects. These findings suggest that the discriminative stimulus effects of mephedrone rely a combination of dopaminergic and serotonergic mechanisms. This conclusion is supported by neurochemical analyses which found that mephedrone administration increases extracellular levels of dopamine and serotonin, as well as the transporter-mediated release of monoamines (Baumann et al., 2012; Hadlock et al., 2011).

The current findings showing partial antagonism with MDL100,907 and pirenperone are generally consistent with previously published results indicating 5-$HT_{2A/C}$ antagonists, ritanserin

and ketanserin, as well as 5-HT$_{2C}$ antagonist, SB242084 all failed to attenuate mephedrone discrimination (Erwin et al., 2019; Saber et al., 2019). Interestingly, in the present study WAY 100, 635 appeared to potentiate the lowest dose of mephedrone (0.375mg/kg). Previous research has established that a low dose (1 mg/kg) of the serotonin reuptake inhibitor, fluoxetine, blocked mephedrone discrimination, while a higher dose (5.6 mg/kg) potentiated mephedrone discrimination (DeLarge et al., 2017). Other neurochemical mechanisms examined in previous studies include glutamatergic and noradrenergic receptor-mediated effects. For example the sigma receptor antagonist rimcazole and the NET inhibitor desipramine were found to potentiate mephedrone (DeLarge et al., 2017). These findings are supported by neurochemical analyses demonstrating substrate activity at 5HT , DA, and NE transporters, while repeated administration of mephedrone produces serotonergic deficits, with no corresponding dopaminergic deficits (Baumann et al., 2012; Hadlock et al., 2011). Considered together, these data suggest that monoamine transporter reuptake inhibition may also play a role in the pharmacological mechanisms underlying the discriminative stimulus effects of mephedrone.

Methodological differences between the current study and previous studies are worth noting. Three of the five aforementioned mephedrone drug discrimination studies utilized cumulative dosing to establish the dose-effect curve (DeLarge et al., 2017; Erwin et al., 2019; Varner et al., 2013). While this method decreases the number of sessions required to produce a dose response curve, it also has been shown to produce quantitative differences from traditional testing and thus may explain disparate results between studies (Thompson et al., 1983). Despite this methodological difference, the results of the present study are generally consistent with other mephedrone discrimination studies that evaluated dopamine and serotonin antagonists.

Another methodological difference between the current study and the previous mephedrone discrimination studies is the use of reinforced test sessions. Aside from Berquist et al. (2017), all of the previous mephedrone discrimination research utilized reinforced test sessions, in which either lever produced reinforcement upon the completion of an FR. This could influence discrimination of the interoceptive effects of mephedrone, though a comprehensive literature review suggests that the drug discrimination assay is robust enough to be unaffected by differences in testing strategies such as reinforced test sessions (Gauvin et al., 2016).

The non-significant reduction in response rates by mephedrone observed in the present study fails to clarify the literature with regard to effects of mephedrone on response rate. Of the five published studies using mephedrone as the training drug, two indicated either no rate reduction or a non-significant rate reduction by mephedrone alone (Berquist et al., 2017; Saber et al., 2019). The remaining three studies reported significant abolishing effects of mephedrone in a dose-dependent fashion (Erwin et al., 2019; Saber et al., 2019; Varner et al., 2013). This difference in effect on response rates could be the result of the dose used, or the training method used to reach the final training dose. The present study and that by Berquist et al.,(2017) began training at the terminal dose (1.0mg/kg or 3.0mg/kg) in contrast to other studies in which the training dose was gradually incremented from 0.5mg/kg until subjects acquired the discrimination or the terminal dose of 3.2mg/kg was obtained (DeLarge et al., 2017; Erwin et al., 2019; Saber et al., 2019; Varner et al., 2013). In a second experiment Saber et al., (2019) implemented a fading procedure by training subjects to discriminate 3.2 mg/kg and gradually reduced the training dose to 0.5 mg/kg. It is unclear whether these differences in training methods led to more sensitive discrimination, or increased sensitivity to the rate decreasing effect

of mephedrone. Direct comparisons between mephedrone dosing methods specifically are

necessary to elucidate these differences.

In summary, the results described herein provide further empirical support for the partial

role of dopamine and serotonin receptors in the discriminative stimulus effects of mephedrone.

These data contribute to a growing body of literature regarding the interoceptive stimulus effects

of mephedrone and serve to inform clinical science regarding the neurochemical mechanisms

involved with abuse risks of this substance. Additional research is necessary to clarify the

neurochemical actions underlying the stimulus effects of mephedrone, which are still not well

understood. Specifically, the role of NET, SERT, sigma receptors and the combination of

dopamine and serotonin receptors should be further evaluated. Moreover, replications with

female subjects are called for, as to date there are no published drug discrimination studies

utilizing female rats trained to discriminate mephedrone.

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APPENDIX

Institutional Animal Care and Use and Committee Letter of Approval

WESTERN MICHIGAN UNIVERSITY

Institutional Animal Care and Use Committee

W

Date: March 27, 2019

To: Lisa Baker, Principal Investigator

From: Kathryn Eckler, Vice Chair

Re: IACUC Protocol Number 19-02-01

Your protocol titled "Drug Discrimination Studies of Psychoactive Drugs in Rats" has received approval from the Institutional Animal Care and Use Committee. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: **March 26, 2020**

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