Published in final edited form as:

Psychopharmacology (Berl). 2019 March ; 236(3): 1015–1029. doi:10.1007/s00213-019-05241-z.

Effects of dopaminergic and serotonergic compounds in rats trained to discriminate a high and a low training dose of the synthetic cathinone mephedrone^a

Iman Saber¹, Andrew Milewski¹, Allen B. Reitz², Scott M. Rawls^{3,4}, and Ellen A. Walker^{1,3}

¹Department of Pharmaceutical Sciences, School of Pharmacy, Temple University, Philadelphia, Pennsylvania, USA

²Fox Chase Chemical Diversity Center, Doylestown, PA,

³Center for Substance Abuse Research, and Temple University, Philadelphia, Pennsylvania, USA

⁴Department of Pharmacology, Lewis Katz School of Medicine, Temple University, Philadelphia, Pennsylvania, USA

Abstract

Rationale—The underlying pharmacological mechanisms of mephedrone, especially as related to interactions with different neurotransmitter systems, are a critical area of study as mephedrone continues to be abused.

Objective—Direct-acting 5-HT_{2A/2C} receptor agonists and antagonists and D_{1–3} receptor antagonists were examined in two groups of rats trained to discriminate mephedrone. A high dose of mephedrone was trained to extend previous results with traditional monoamine transporter inhibitors and substrate releasers. A very low dose of mephedrone was trained to preferentially capture serotonergic activity and to minimize the influence of rate-decreasing effects on substitution patterns. Selective 5-HT_{2A/2C} and D_{1–3} receptor antagonists were examined in both groups.

Methods—Male, Sprague-Dawley rats were trained to discriminate either a low dose of 0.5 mg/kg mephedrone (N=24) or a high dose of 3.2 mg/kg mephedrone (N= 11) from saline.

Results—In the low training-dose group, mephedrone, MDMA, methamphetamine, *d*-amphetamine, cocaine, and enantiomers of mephedrone substituted for mephedrone; mCPP partially substituted overall for mephedrone; and, DOI, WAY163909, and morphine failed to substitute for mephedrone. In the high training-dose group, only mephedrone and MDMA substituted for mephedrone. Sulpiride produced a small antagonism of the low training dose of mephedrone while SCH23390, SB242084, and ketanserin altered response rates.

Conclusions—A lower training dose of mephedrone produces a discriminative stimulus fully mimicked by MDMA, methamphetamine, cocaine, and *d*-amphetamine whereas a higher training

^aThis project was conducted in partial fulfillment of the doctorate degree in Pharmaceutical Sciences at Temple University for Iman Saber.

Correspondence to Ellen A Walker, PhD, Department of Pharmaceutical Sciences, School of Pharmacy, Temple University, 3307 North Broad Street, Philadelphia, PA 19140, USA, ellen.walker@temple.edu.

dose of mephedrone requires a discriminative stimulus that was only mimicked by MDMA. Dopaminergic or serotonergic antagonists failed to produce significant blockade of mephedrone at either training dose.

Keywords

drug discrimination; ketanserin; MDMA; mephedrone; methamphetamine; psychostimulants; rats; SB242084; SCH23390; sulpiride

Mephedrone is a ring-substituted synthetic cathinone (e.g., Simmons et al.2018; Zaami et al. 2018) that inhibits dopamine, norepinephrine and serotonin reuptake by acting as a substrate at DAT, NET and SERT (Baumann, et al.2018, 2012; Kehr et al.2011; Martinez-Clemente et al.2012; Suyama *et al.*2016; Hadlock *et al.*2011). Depending on the studies, mephedrone either has similar potencies at DAT and SERT (Baumann et al.2012; Hadlock et al.2011; Simmler et al.2013), greater potency at DAT relative to the SERT (Eshleman et al.2013), or greater potency at the NET than the DAT (Luethi et al.2018; Simmler et al.2013) with a DAT/SERT ratio of 2.4 (Bonano et al.2015). Using in vivo measurements, mephedrone increases extracellular serotonin levels in a manner similar to MDMA (Baumann et al.2012; Kehr et al.2011; Suyama et al.2015) yet also increases extracellular dopamine in a manner similar to d-amphetamine (Kehr et al.2011). In radioligand binding studies, mephedrone can exhibit affinity for the 5-HT_{2A}, 5-HT_{2C}, 5-HT_{1A}, α_{1A} , α_{2A} , and TAAR receptors (Eshleman et al.2013; Simmler et al.2013; Rickli et al.2015a; Luethi et al.2018). Behaviorally, similar to MDMA and methamphetamine, mephedrone produces conditioned place preference (Lisek et al.2012), intracranial self-stimulation (Suyama et al.2015), and maintains self-administration in rats (e.g., Aarde et al.2013; Creehan et al.2015) demonstrating that mephedrone serves as a reinforcer.

To further delineate the underlying pharmacological mechanisms responsible for the behavioral effects of mephedrone relative to other abused stimulants, previous investigators have used drug discrimination assays (Berquist II et al. 2017; DeLarge et al.2017; Gannon and Fantegrossi, 2016; Gatch et al.2013; Harvey et al.2017; Harvey and Baker, 2016; Smith et al.2016; Varner et al.2013). Indeed the drug discrimination assay has proven exceptionally useful for characterizing the interoceptive effects and underlying pharmacological mechanisms for monoamine reuptake inhibitors such as cocaine and for monoamine substrate/releasers such as MDMA (for review see Berquist II and Fantegrossi, 2018). In rats trained to discriminate either methamphetamine or cocaine, mephedrone fully substituted for both methamphetamine and cocaine (Gatch et al.2013). When rats were trained to discriminate between d-amphetamine and saline, mephedrone fully substituted for d-amphetamine and the D₁ antagonist SCH39166 blocked this substitution suggesting that these D₁ receptors were crucial for the capacity of mephedrone to substitute for d-amphetamine (Harvey et al.2017). When rats were trained to discriminate mephedrone, cocaine, and methamphetamine produced partial to almost full substitution (Varner et al. 2013) and the patterns and extent of substitution and cross-substitution were dependent on mephedrone training doses (Berquist II, et al. 2017). Overall, these studies suggest that mephedrone produces discriminative stimulus effects similar to stimulants that reveal

predominantly dopamine (methamphetamine, cocaine) and 5-HT (MDMA) underlying pharmacological mechanisms.

Few of the studies described above, however, have tested direct-acting agonists or specific receptor selective dopamine or 5-HT antagonists. LSD, which has affinity at many 5-HT subtypes including 5-HT_{2A} receptors, α_1 receptors, and D₁₋₃ receptors (Rickli et al.2015b), produced partial substitution for low and high training doses of mephedrone (Berquist II et al.2017) and DOI, which has higher selectivity for 5-HT_{2A} over 5-HT_{2C} receptors (Pigett et al.2012), produced partial substitution for a high dose of mephedrone in a small portion of the rats before rate-decreasing effects impacted testing (DeLarge et al.2017). Attempts to block the discriminative stimulus effects of mephedrone with haloperidol (Varner et al.2013) or the sigma antagonist rimcazole (DeLarge et al.2017) have met with limited success. Currently, little information is available on the susceptibility of the discriminative stimulus or rate-decreasing effects of mephedrone to antagonism by more selective dopamine antagonists or any 5-HT antagonists in mephedrone-trained subjects.

Therefore, in the current study, we trained one group of rats to discriminate a high dose of 3.2 mg/kg mephedrone from saline and another group of rats to discriminate a very low training dose of 0.5 mg/kg mephedrone from saline using two-choice drug discrimination procedures. Thereafter, we tested a collection of similar nonselective monoamine substrate releasers and inhibitors of dopamine and 5-HT transport as previously reported (Varner et al. 2013; Berquist et al. 2017; DeLarge et al.2017) and expanded our substitution tests to include 5-HT_{2C} receptor agonist WAY16909, 5-HT_{2A} receptor agonist DOI (Zea-Ponce et al.2002), and nonselective 5HT_{2C}/5-HT_{1B} agonist mCPP to investigate role of these 5-HT receptor subtypes in the discriminative stimulus effects of mephedrone of either a high or low training dose of mephedrone. In addition, we examined multiple doses of D₁ receptor antagonist SCH23390, D_{2/3} receptor antagonist sulpiride, 5-HT_{2C} receptor antagonist SB242084, and 5HT_{2A/2C} antagonist ketanserin alone and as pretreatments to multiple doses of mephedrone to determine if these antagonists would substitute for or block mephedrone. We hypothesized that drugs with SERT inhibition, serotonin substrate release, and/or direct acting receptor activity for serotonin receptors, especially 5-HT_{2A} and 5-HT_{2C} receptors, would preferentially substitute for the lower training dose of mephedrone and these effects of mephedrone would be blocked by SB242084 and ketanserin. At a higher training dose of mephedrone, however, we predicted that more dopaminergic activity would be recruited for the discriminative stimulus effects of mephedrone and drugs with relatively non selective monoamine transporter inhibition and substrate/release would substitute preferentially and both serotonin and dopamine receptor antagonists would block the effects of mephedrone in the higher training dose group.

Materials and Methods

Subjects

Naive, male Sprague-Dawley rats (Taconic Biosciences, Inc., Cranbury, NJ) weighing 200–250g, at the start of the experiment, were initially group-housed under a reversed 12-h light/dark cycle with water available *ad libitum* (N=35) for a two week acclimation period. During the experiments, rats were individually housed, placed on a restricted food diet, and

maintained at approximately 85% of their free feeding body weights by earning pellets in the experimental chambers and receiving approximately 15 g of Purina Rodent Chow each day after the session. All rats were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee of Temple University (Institution of Laboratory Animal Research, National Academy Press; Eighth edition, revised 2011).

Drugs

The following drugs and doses were tested for their capacity to substitute for the discriminative stimulus effects of mephedrone: 0.5–15 mg/kg cocaine, 0.15–3.0 mg/kg *d*-N-methylamphetamine (methamphetamine), 0.03125–2.0 mg/kg *d*-amphetamine, 0.1–2.0 mg/kg 2,5-dimethoxy-4-iodoamphetamine (DOI), 0.5–9.0 mg/kg 3,4-methylenedioxymethamphetamine (ecstasy; MDMA), 0.3–1.6 mg/kg 1-(*m*-chloro phenyl)piperazine (meta-chlorophenylpiperazine; mCPP), 0.05–1.0 mg/kg WAY163909 [(7*b*-R,10*a*-R)-1,2,3,4,8,9,10,10*a*-octahydro-7*b*H-cyclopenta[*b*][1,4] diazepino [6,7,1*hi*] indole], 0.28–5.0 mg/kg morphine, 0.5–1.6 mg/kg R-mephedrone, 0.05–0.5 mg/kg S-mephedrone. The following antagonists and doses were tested for the capacity to block the discriminative stimulus and rate-decreasing effects of mephedrone: D₁ receptor antagonist SCH23390 (0.0125–0.06 mg/kg), the D_{2/3} receptor antagonist sulpiride (2.0–4.0 mg/kg), the non-selective 5-HT_{2A/2C} receptor antagonist ketanserin (1.0–1.5 mg/kg), and the selective 5-HT_{2C} receptor antagonist SB242084 (0.5–1.0 mg/kg).

Mephedrone hydrochloride, S-mephedrone hydrochloride, and R-mephedrone hydrochloride were provided to Dr. Rawls by Fox Chase Chemical Diversity Center, Inc. (Doylestown, PA, USA) as previously described (Gregg et al.2015). The National Institute on Drug Abuse Drug Supply Program (Rockville, MD) provided cocaine, morphine, methamphetamine, and d-amphetamine. MDMA was purchased from Sigma-Aldrich (St. Louis, MO). DOI, mCPP, and the receptor antagonists, ketanserin, SCH23390, sulpiride, and SB242084 were purchased from Tocris Bioscience (Ellisville, MO). Wyeth Pharmaceuticals (Princeton, NJ) generously donated WAY163909. Mephedrone, mCPP, cocaine, morphine, methamphetamine, d-amphetamine, MDMA, DOI, and SCH23390 were each dissolved in 0.9% saline. Ketanserin was dissolved in 0.9% saline and sonicated for approximately 1 h. Sulpiride was first dissolved in ethanol and then titrated to a final concentration using 0.9% saline and a few drops of 8% lactic acid were used to neutralize the pH. SB242084 was first dissolved in 5% DMSO and titrated to final concentration with 0.9% saline. All injections were given intraperitoneally (i.p.) in a volume of 0.5 or 1.0 mL/kg of body weight.

Apparatus

Experiments were conducted in 12 operant experimental chambers (Model ENV-008CT, Med Associates, Inc., St. Albans, VT, USA) located within ventilated sound attenuating enclosures (ENV-018MD) located in a designated room. Each chamber featured, on one wall, two amber stimulus lights (Model ENV-221M) located directly above two retractable levers (Model ENV-112CM) positioned 2.1 cm above the stainless steel grid floor and 7.62 cm apart from one another, a center receptacle located between the two levers, and a pellet feeder (Model ENV-200R2M). A house light and ventilator fans were located on the

opposite wall. Experimental contingencies and data collection were controlled using a computer-driven interface (Model SG-503, MED Associates, St. Albans, VT, USA).

Procedure

Rats were initially trained to respond on two levers, on alternate days, on a fixed ratio 1 (FR1) schedule of banana-flavored, sucrose pellet delivery (45 mg, BioServ, Flemington, NJ). Once rats were reliably responding on each lever to earn 50 reinforcers after approximately two weeks, rats were injected i.p. with either saline or a training dose of mephedrone and placed in the experimental chamber. After a 10 min timeout, the house and stimulus lights were illuminated and the levers were inserted into the chamber. Rats were trained to discriminate mephedrone on the left lever and saline on the right lever on a gradually increasing schedule from FR1 to FR10. Responding on the inappropriate lever was not reinforced and reset the ratio requirement. Each trial lasted for 10 reinforcers or 5 min, whichever occurred first. After stable FR10 responding, rats were moved to a two-trials training procedure which included a 10 min timeout, followed by a 5 min ratio component, followed by a second 10 min timeout period and a second 5 min ratio component. The purpose of the two-trials training procedure was to expose the rats more frequently to different training stimuli and to prepare the rats for testing in the antagonism studies in which the antagonist dose was tested alone in the first trial. Two saline trials, two mephedrone trials (2nd injection was saline but the mephedrone lever was reinforced in both trials), or a saline then a mephedrone trial were the possible combinations of two trial training sessions. No injection (saline or mephedrone) was administered for more than three consecutive trials across two training days.

Rats were assigned to either the low training dose group or the high training dose group. The high dose training group (n= 11) was trained to discriminate the final training dose of 3.2 mg/kg mephedrone from saline. This group initially started at a training dose of 0.5 mg/kg mephedrone which was then gradually increased (0.5→2.0→2.5→3.2 mg/kg) until a final training dose of 3.2 mg/kg mephedrone was obtained. Each time the training dose was incremented, the rats would continue to train at that new higher dose for one or two weeks until response rates were within approximately 20% of the saline control response rates. Two groups of rats (n=12 each) were trained to discriminate a dose of 0.5 mg/kg mephedrone from saline. Of these 24 rats, the first group of 12 rats initially began training at a higher dose of 3.2 mg/kg mephedrone and the dose was gradually decremented (3.2→1.0→0.5) to a final dose of 0.5 mg/kg mephedrone although starting at the higher doses produced a disruption of response rates. Each time the training dose was decreased, the rats would continue to train at that new lower dose for one to three weeks with the goal of obtaining response rates similar to the saline control response rates. The second group of 12 rats began initial training with the low training dose of 0.5 mg/kg mephedrone. Rats were trained until seven consecutive training trials (~3.5 days of two trial each) were met with the following criteria: 1) fewer than 10 responses on the inappropriate lever before the first reinforcer; and, 2) greater than 80% of the injection appropriate responding over the entire training trial. Additional testing criteria were that both single (SMS or MSM) and double alternation (MM, SS) training trials were required to be correct according to the above testing criteria. Once all criteria were met, testing began.

Test trials were identical to the two training trials except the test compound was injected before the trial instead of the training injections and a complete FR10 on either lever was reinforced. When testing for substitution, the first trial was either the test dose or saline and the second trial was either a saline injection or a test dose, respectively, to maintain the two trial procedure as similar to the training procedure. If the rat failed to respond according to the above testing criteria after a saline injection in the first trial under test conditions, the test was stopped and training re-instituted. During antagonism tests, the dose of antagonist was administered in the first trial and the test dose of mephedrone was administered in the second trial. After each test session, rats had to meet the testing criteria for two or three consecutive training trials including both mephedrone and saline trials before testing again.

Data Analysis

The percentage of lever selection during training or testing was determined by dividing the responses made on the mephedrone lever by the total responses made on both levers during the trial. Response rate was measured as responses on both levers during the trial divided by the total seconds of that trial. For individual as well as grouped data, full substitution was defined as >80% responding on the mephedrone-appropriate lever, partial substitution was defined as between 20%–80% responding on the mephedrone-appropriate lever, and responding below 20% was considered to not substitute for the training dose of mephedrone. Effects at each dose of drug were expressed as a group mean, along with the standard error of the mean (S.E.M.). The data from rats that failed to complete one full ratio (i.e., 10 responses) were included in the response rate but not the discrimination data. For each dose or dose combination, the numbers of rats included in the discrimination data and the total number of rats tested are listed in the figure legends. Dose-response curves for the discriminative stimulus effects of mephedrone, methamphetamine, R-mephedrone, and S-mephedrone were analyzed by the linear regression to determine ED₅₀ values and 95% C.L. (GraphPad Prism 7.0 Software, Inc, La Jolla, CA).

A repeated measures, one-way ANOVA was used to determine if response rates varied by dose; if so, the ANOVA was followed by Dunnett's Multiple Comparison tests to determine which dose of test drug significantly changed response rates. To examine the dose-dependent effects of a given antagonist, a mixed, repeated measures one-way ANOVA, followed by Dunnett's Multiple Comparison tests was used including each dose of antagonist and the corresponding data from training dose of mephedrone for every rat tested with a dose of antagonist (GraphPad Prism 7.0 Software, Inc, La Jolla, CA). Significance was reported when the analyses reached a significance level of at least $p < 0.05$.

Results

Training and potency comparisons

For the high dose training group ($n = 11$), initial discrimination training began using a dose of 0.5 mg/kg mephedrone and gradually increased over 94 ± 81 trials (~47 days) until a final dose of 3.2 mg/kg was attained. Thereafter, 10 rats acquired the mephedrone vs. saline discrimination after 88 ± 30 trials (~44 days). One rat failed to discriminate after 330 trials (~5 months) and was transferred to the low training dose group and completed training after

59 trials (~30 days). One rat that failed to learn to discriminate 0.5 mg/kg mephedrone from saline after 250 trials (~4 months) was transferred to the high dose group and then completed training after 35 trials (~17 days). For the 0.5 mg/kg mephedrone group (Group 1, n=12; Group 2, n=12), the initial training dose for mephedrone was 3.2 mg/kg for Group 1. However, this dose initially decreased response rates to less than 0.2 responses/s. The mephedrone training dose was reduced to 1.0 and then 0.5 mg/kg over the course of 8 weeks. Group 2 began initial discrimination training at a dose of 0.5 mg/kg mephedrone. Once all rats were at the final training dose of 0.5 mg/kg mephedrone, an average of 101 trials (~50 days) were required to acquire the discrimination.

In the high dose training group, when saline was administered during test trials, responses were directed to the saline-appropriate lever (Figure 1, left panels). Increasing doses of mephedrone produced dose-dependent increases in mephedrone-appropriate responding and decreases in response rates. Higher doses of mephedrone produced full substitution for the training dose. Overall response rates were significantly decreased after mephedrone ($F(6,62)=5.8$; $p<0.0001$) and post-hoc tests indicated the highest dose of 5.0 mg/kg mephedrone significantly decreased rate of responding compared to saline ($p<0.003$). Morphine (0.3–5.0 mg/kg) did not substitute for the 3.2 mg/kg mephedrone in any rats although the response rates were significantly decreased ($F(3,27)=7.13$; $p<0.001$). Specifically, 5.0 mg/kg morphine decreased response rates relative to saline ($p<0.003$).

In the low training dose group, saline produced saline-appropriate responding and mephedrone produced dose-dependent increases in mephedrone-appropriate responding and significant overall decreases in response rates ($F(4,108)=2.72$; $p<0.03$). However, post-hoc tests did not indicate specific doses of mephedrone as different from saline (Figure 1, right panels). Morphine produced substitution for 0.5 mg/kg mephedrone training dose in only 2/10 rats tested and significantly decreased overall response rates ($F(3,53)=2.91$; $p<0.04$) although post-hoc tests did not indicate specific doses of morphine as different from saline. The mephedrone enantiomers were also tested in the rats trained to discriminate 0.5 mg/kg mephedrone from saline. S-Mephedrone fully substituted for mephedrone in 11/12 rats and significantly increased response rates ($F(3,54)=6.4$; $p<0.001$); post hoc tests indicated that 0.05 and 0.15 mg/kg produced higher response rates than saline ($p<0.001$ and $p<0.003$, respectively). R-mephedrone substituted completely in 12/12 rats and also significantly increased response rates $F(2,43)=6.67$; $p<0.003$); post hoc tests indicated both 0.5 and 1.5 mg/kg R-mephedrone increased response rates relative to saline ($p<0.005$ and $p<0.01$, respectively).

The ED_{50} value $\pm$ 95% C.L. for mephedrone in the high training dose group was 0.91 mg/kg (0.7–1.3) and the ED_{50} value $\pm$ 95% C.L. for mephedrone in the lower training dose group was 0.23 mg/kg (0.12–0.44), about 4-fold less potent than in the higher training dose group. Despite different training histories, the two groups in the 0.5 mg/kg training dose groups had similar dose-response curves for mephedrone such that the ED_{50} values $\pm$ 95% C.L. for mephedrone were 0.25 mg/kg (0.19–0.32) in Group 1 and 0.15 mg/kg (0.089–0.23) in Group 2. In the 0.5 mg/kg mephedrone training dose group, the ED_{50} value $\pm$ 95% C.L. for the R-mephedrone enantiomer was 0.71 mg/kg (0.54–0.87) while the S-mephedrone was 5-fold more potent with an ED_{50} value $\pm$ 95% C.L. of 0.14 mg/kg (0.073–4.2).

Cocaine, d-amphetamine, and methamphetamine substitution tests in the high and low training dose groups—

In the high training dose group, methamphetamine substituted at some dose in 5/8 rats (63%) and partially substituted in 1/8 rats while cocaine substituted in 3/9 rats (30%) at some dose and partially substituted in 1/9 rats resulting in overall partial substitution for the group. D-amphetamine produced substitution in 2/8 rats at a low dose producing only 18% mephedrone-appropriate responding for the group. At higher doses of amphetamine, these rats responded on the saline lever and one additional rat responded partially on the mephedrone lever (Figure 2; left panels). The drugs d-amphetamine ($F(7,46)=4.24$; $p<0.001$) and methamphetamine ($F(5,40)=3.707$; $p<0.007$) produced overall response rates significantly different than baseline saline response rates according to one-way ANOVA but post hoc analyses did not reveal individual dose differences. The doses of cocaine tested did not alter response rates significantly from saline values and higher doses were not tested due to concerns for toxicity.

In the low training dose group, all 8 rats tested with the higher doses of 10 and 15 mg/kg cocaine substituted completely for 0.5 mg/kg mephedrone (Figure 2, right panels). Lower doses of 0.5, 0.9, and 5 mg/kg cocaine tested in 12 rats produced a wide range of mephedrone-appropriate responding between 0 and 98%. Methamphetamine fully substituted in 20/21 rats, and d-amphetamine fully substituted in 19/20 rats at some dose. One-way ANOVA revealed overall changes in response rates for cocaine ($F(5,68)=3.74$; $p<0.05$), d-amphetamine ($F(7,82)=8.79$; $p<0.0001$), and methamphetamine ($F(5,89)=11.25$; $p<0.0001$). Specifically, post-hoc tests indicated doses of 0.5 and 0.9 mg/kg of cocaine ($p<0.05$ and $p<0.05$) and 0.3 mg/kg d-amphetamine ($p<0.05$) increased response rates while doses of 1.0 and 2.0 mg/kg d-amphetamine ($p<0.05$ and $p<0.001$, respectively) and 3.0 mg/kg methamphetamine ($p<0.0001$) decreased response rates compared to saline. Despite different training histories, the two groups in the 0.5 mg/kg training dose groups had similar dose-response curves for methamphetamine such that the ED_{50} values + 95% C.L. for methamphetamine were 0.18 mg/kg (0.11–0.24) in Group 1 and 0.20 mg/kg (0.030–0.32) in Group 2.

DOI, WAY16390, MDMA, and mCPP in high and low training dose groups—In the high training dose group, DOI partially substituted for 3.2 mg/kg mephedrone in only 2/8 rats tested. One-way ANOVA indicated significant overall changes in response rates for the DOI dose-response curve ($F(4,31)=2.73$; $p<0.05$) yet post hoc tests revealed that no individual doses were different from saline control rates (Figure 3; left panels). WAY163909 only partially substituted for the training dose of 3.2 mg/kg mephedrone in 1/6 rats tested; however, WAY163909 significantly reduced response rates ($F(5,34)=7.16$; $p<0.0001$), specifically a dose of 1.0 mg/kg WAY163909 ($p<0.01$). MDMA produced full substitution for 3.2 mg/kg mephedrone in 8/9 rats at some dose tested. One-way ANOVA revealed that MDMA disrupted response rates ($F(4,35)=8.18$; $p<0.001$) and post hoc tests indicated a dose of 10 mg/kg MDMA significantly decreased response rates ($p<0.003$) compared to saline. The drug mCPP was not tested in this group of rats.

In the lower 0.5 mg/kg mephedrone training dose group, DOI produced full substitution in 4/9 rats and partial substitution in 1/5 rats which resulted in overall intermediate partial

substitution for the group (Figure 3; right panels). However, a lot of variability was observed for the dose of DOI to decrease response rates for individual rats which limited testing higher doses in all rats. One-way ANOVA revealed that DOI significantly decreased response rates ($F(4,51)=12.1$; $p<0.0001$) and post hoc tests indicated that 0.5, 1.0, and 2.0 mg/kg DOI significantly decreased response rates relative to saline response rates ($p<0.001$, $p<0.0001$, $p<0.0001$, respectively). WAY163909 produced substitution in 2/12 rats and partial substitution in 3/12 rats at some dose resulting in overall low partial substitution for the group. Similar to the results with DOI, a lot of variability was observed for the dose of WAY163909 that decreased response rates for individual rats. One-way ANOVA revealed that WAY163909 significantly disrupted responding ($F(5,58)=16.39$; $p<0.0001$) and post hoc tests indicated that 0.5, 0.75 and 1.0 mg/kg significantly decreased response rates ($p<0.0001$). MDMA produced full substitution at some dose in 12/12 rats. One-way ANOVA revealed significant differences for the different doses of MDMA to produce changes in response rates ($F(4,64)=12.67$; $p<0.0001$). Specifically, post hoc tests indicated that 0.5 mg/kg MDMA increased responding ($p<0.01$) while 5.0 mg/kg MDMA ($p<0.0001$) decreased rates of responding compared to saline. The agonist mCPP fully substituted in 8/12 rats at one or both of the two lower doses of mCPP and an additional rat produced 76% mephedrone-appropriate responding after the highest dose of mCPP. Three of these rats, however, only partially substituted for 0.5 mg/kg mephedrone at the highest dose of mCPP which resulted in a flatter dose-response curve when the data were averaged together in the group. One-way ANOVA indicated that mCPP decreased response rates ($F(3,54)=17.93$; $p<0.0001$) and post hoc tests revealed a dose of 1.5 mg/kg mCPP significantly decreased response rates compared to saline ($p<0.0001$).

D₁ and D_{2/3} Receptor Antagonist Tests—In the high training dose group, doses of SCH23390 alone decreased the response rates as described by one-way ANOVA ($F(3,35)=14.78$; $p<0.0001$) (Figure 4, left panels). Post hoc tests indicated that 0.0125, 0.025, and 0.06 mg/kg SCH23390 alone decreased response rates relative to saline test response rates ($p<0.0001$, $p<0.001$, and $p<0.0001$, respectively). In combination with the training dose of 3.2 mg/kg mephedrone, SCH23390 reduced response rates ($F(3,21)=19.06$; $p<0.0001$); post hoc tests indicated that doses of 0.0125, 0.025, and 0.06 mg/kg SCH23390 in combination with 3.2 mg/kg mephedrone decreased response rates relative to 3.2 mg/kg mephedrone alone ($p<0.0001$, $p<0.001$, and $p<0.0001$, respectively). These rate-decreasing effects prevented the collection of meaningful discrimination data. In the low training dose group, SCH23390 blocked the discriminative stimulus effects of 0.5 mg/kg mephedrone ($F(2,18)=4.412$; $p<0.05$), specifically a dose of 0.025 mg/kg SCH23390 ($p<0.05$) (Figure 4, right panels). SCH23390 reduced response rates relative to saline ($F(3,73)=29.93$; $p<0.0001$), specifically doses of 0.025 and 0.06 mg/kg SCH23390 alone disrupted response rates relative to saline ($p<0.0001$ and $p<0.0001$, respectively). Overall response rates were significantly decreased ($F(3,36)=24.79$; $p<0.0001$) and doses of 0.025 and 0.06 mg/kg SCH23390 in combination with 0.5 mg/kg mephedrone decreased response rates relative to 0.5 mg/kg mephedrone alone ($p<0.0001$; $p<0.0001$, respectively). At the training dose of 0.5 mg/kg mephedrone, only 3/10 and 1/11 rats responded in combination with 0.025 and 0.06 mg/kg SCH23390, respectively. Again, these rate-decreasing effects of SCH23390 alone and in combination with mephedrone render the discrimination data difficult to interpret.

In the high dose training group, no dose of sulpiride blocked the discriminative effects or the rate-decreasing effects of 3.2 mg/kg mephedrone (Figure 5; left panels). However, sulpiride alone increased the response rates relative to saline ($F(2,24)=3.56$; $p<0.05$) specifically a dose of 4.0 mg/kg sulpiride ($p<0.05$). In rats trained to discriminate 0.5 mg/kg mephedrone, however, an overall effect of sulpiride on mephedrone discrimination of the training dose was observed ($F(2,29)=4.153$; $p<0.05$). Post hoc tests indicated that a dose of 2.0 mg/kg sulpiride significantly reduced 0.5 mg/kg mephedrone-appropriate responding ($p<0.05$) (Figure 5; right panels). One-way ANOVA revealed significant response rate changes for sulpiride pretreatment ($F(2,30)=7.071$; $p<0.05$). Post hoc test indicated that 2.0 mg/kg sulpiride pretreatment in combination with the training dose of 0.5 mg/kg mephedrone produced an overall increase in response rates relative to 0.5 mg/kg mephedrone alone ($p<0.05$).

5-HT_{2c} and 5-HT_{2A/2c} Receptor Antagonist Tests—In the high dose training group, no dose of SB242084 blocked the discriminative effects or the rate-decreasing effects of 3.2 mg/kg mephedrone (Figure 6, left panels). However, SB242084 alone increased the response rates relative to saline ($F(2,23)=4.29$; $p<0.05$), specifically the dose of 1.0 mg/kg SB242084 ($p<0.05$). In the low training dose group, no dose of SB242084 altered the discriminative stimulus effects of mephedrone; however, SB242084 did increase response rates when combined with mephedrone ($F(2,27)=4.881$; $p<0.05$) (Figure 6, right panels). Post hoc tests revealed that the dose of 0.5 mg/kg SB242,084 in combination 0.5 mg/kg mephedrone increased response rates relative to 0.5 mg/kg mephedrone alone ($p<0.05$). In the high dose training group, no dose of ketanserin altered either the discriminative stimulus or rate-decreasing effects of mephedrone (Figure 7, left panels). In the low training dose group, both doses of ketanserin produced a small, but insignificant shift in the discriminative stimulus effects of mephedrone (Figure 7, right panels). However, ketanserin in combination with the training dose of 0.5 mg/kg mephedrone produced a significant increase in response rates relative to 0.5 mg/kg mephedrone alone ($F(2,24)=4.094$; $p<0.05$), specifically the dose of 1.5 mg/kg ketanserin in combination 0.5 mg/kg mephedrone increased response rates relative to 0.5 mg/kg mephedrone alone ($p<0.05$).

Discussion

In the present study, high and low training doses of mephedrone were established as discriminative stimuli in two sets of rats. The higher dose of 3.2 mg/kg mephedrone has been trained previously in rats (Varner et al.2013; DeLarge et al.2017) but we extended the training dose literature to include a 6-fold lower training dose of 0.5 mg/kg mephedrone, currently the lowest dose of mephedrone successfully trained as a discriminative stimulus. The time to testing criteria and ease of training these two training doses were different between the two groups mostly due to the behaviorally disruptive effects of higher doses of mephedrone and the time required to either decrease or increase the training doses. Nevertheless, two training trials per day doubled the opportunity for the rats to learn the two different discriminative stimuli, saline vs. mephedrone, so overall the days to criterion were similar to previous studies (Varner et al.2013). The 0.5 mg/kg training dose group was composed of two sets of rats: the first group started at a dose of 3.2 mg/kg mephedrone and

this dose was decreased to 0.5 mg/kg mephedrone over a series of 8 weeks; and, the second group started training a year later initially with 0.5 mg/kg mephedrone. Acquisition of the discrimination required fewer training sessions when starting directly with 0.5 mg/kg mephedrone. Despite the different training histories, however, mephedrone and methamphetamine, two compounds tested in all the rats, were equipotent in both sets of 0.5 mg/kg trained rats. There were no other obvious differences between these two groups for any of the other data collected. Both of the mephedrone enantiomers produced full substitution for the 0.5 mg/kg mephedrone discriminative stimulus which is not surprising considering the racemic mixture was used for training.

The R-mephedrone enantiomer was approximately 5-fold less potent than S-mephedrone and interestingly, both of the enantiomers increased response rates at most of the doses tested. Prior work directed toward stereochemical separation of mephedrone pharmacology has found marked differences in the mephedrone enantiomers in preclinical assays. S-mephedrone does not produce conditioned place preference in rats and, relative to R-mephedrone, displays less locomotor activation, reinforcing efficacy in progressive-ratio self-administration assays, and facilitation of intracranial self-stimulation (Gregg et al.2015; Philogene-Khalid et al.2017a). In addition, S-mephedrone reduces the anxiogenic and depressant effects precipitated by abstinence from chronic cocaine or MDPV (methylenedioxypyrovalerone) (Philogene-Khalid et al.2017b). At the neurochemical level, both mephedrone enantiomers display similar effects on dopamine release, but R-mephedrone is much weaker in its ability to release 5-HT. In fact, using the DAT/SERT ratio, a metric that defines preference for drug-induced release effects at dopaminergic neurons over 5-HT neurons, R-mephedrone displays a 50-fold greater preference for the dopaminergic system than S-mephedrone (Gregg et al.2015). This neurochemical signature (i.e., S-mephedrone being a relatively stronger 5-HT releaser) may have impacts on our drug discrimination outcomes especially in consideration of the different transporter inhibitors and substrate/releasers tested for substitution in the current study.

For example, MDMA was the only compound besides mephedrone tested in the current study that produced full substitution in both the low and high training dose groups of mephedrone. Our results are similar to previous studies in which full substitution or near full substitution of similar doses of MDMA was observed when using 1.0 mg/kg and 3.0 or 3.2 mg/kg mephedrone as training stimuli (Berquist II et al. 2017; Varner et al.2013). Despite the different training and testing procedures used in the current study, these common substitution patterns with MDMA across the studies demonstrate the reproducibility of the drug discrimination procedure. When rats were trained to discriminate 1.5 mg/kg MDMA or a combination of 1.5 mg/kg MDMA + 0.5 mg/kg d-amphetamine, mephedrone fully substituted for MDMA in both groups (Harvey and Baker 2016). Similar to MDMA, mephedrone inhibits SERT, DAT, and NET and functions nonselectively as a substrate for release at these transporters with greater release of 5-HT (Baumann et al.2012; Kehr et al. 2011; Luethi et al.2018) so it is not surprising that reciprocal cross-substitution would be observed between mephedrone and MDMA. In general, the discriminative stimulus effects of MDMA consists of both serotonergic and dopaminergic components (for review see Berquist II and Fantegrossi 2018) and drugs that bind directly to 5-HT receptors such as

LSD and DOM can produce MDMA-like discriminative stimulus effects (Goodwin et al. 2003).

Indeed, the observation that mephedrone inhibits SERT, increases serotonin release and can bind to 5-HT_{2A} and 5-HT_{2C} receptors in radioligand binding assays (Eshleman et al.2013; Luethi et al.2018), suggests that direct-acting 5-HT receptor agonists for these receptors might share discriminative stimulus effects with mephedrone as seen previously with MDMA (Goodwin and Baker 2000; Goodwin et al.2003). However, less than half of the rats tested with the 5-HT_{2A} receptor agonist DOI and 5-HT_{2C} receptor agonist WAY163909 produced full mephedrone responding in the low training dose group and essentially no substitution for these agonists was observed in the high training dose group. DOI and WAY163909 produced significant rate-decreasing effects which limited testing higher doses, however. The DOI results are similar to that observed in previous studies, i.e., low to intermediate mephedrone-appropriate responding before a marked reduction in response rates (DeLarge et al.2017). Similarly, DOI only partially substituted for MDMA and produced substantial rate-decreasing effects (Goodwin and Baker 2000). The nonselective 5-HT_{2C/1B} receptor agonist mCPP produced more substitution for mephedrone in most of the twelve rats tested in the low training dose group and less rate-decreasing effects in comparison to the more selective compounds WAY163909 and DOI. Although more individual substitution was observed for mCPP than for WAY163909 and DOI in the low training dose group, the mCPP dose-response curve was somewhat flat due to the usual pattern of more substitution at lower doses of mCPP for some rats resulting in an overall partial substitution result for the averaged group data. Although mCPP possesses binding affinity at most 5-HT receptors and interacts with SERT (Roth and Driscoll 2018; Pettibone and Williams 1984), the discriminative stimulus effects of mCPP appear to be mediated predominantly through 5-HT_{2C} receptors (Callahan and Cunningham 1994; Gommans et al. 1998). However, the likelihood that mCPP produces mephedrone-like responding solely through 5-HT_{2C} receptors is improbable given the lack of substitution of WAY163909. More likely mCPP produces more mephedrone substitution at some dose than WAY163909 because mCPP binds to many 5-HT receptors and mCPP can inhibit SERT and can cause the release of serotonin as well (Pettibone and Williams 1984). LSD, an agonist with a wide range of 5-HT_{2A}, 5-HT_{2C}, 5-HT_{1A}, and D₁₋₃ receptor binding affinities and no interactions with monoamine transporters (Rickli et al.2015b; Roth and Driscoll 2018), produced partial substitution for 1 or 3 mg/kg mephedrone in rats although response rates were very low (Berquist II et al. 2017). When examined together, these substitution data for the direct-acting 5-HT receptor agonists in rats trained to discriminate mephedrone indicate no activation of a single 5-HT receptor per se, but likely a pattern of broad-based receptor activation is needed especially for the low training dose of mephedrone. The observation that neither DOI or WAY163909 in the current study or LSD (Berquist II et al. 2017) or DOI (DeLarge et al.2017) in previous studies substituted for the higher doses of mephedrone suggests that the direct activation of serotonin receptors is not necessary for these discriminative stimulus effects.

Other less selective drugs such as cocaine, a monoamine transporter inhibitor, and methamphetamine and d-amphetamine, monoamine transporter substrate/releasers, produced partial or full substitution in the high and low training dose groups, respectively, with overall

similar results to previous studies (Varner et al.2013; Berquist II et al.2017; DeLarge et al. 2017). Although mephedrone shares dopamine, serotonin, and norepinephrine substrate release with methamphetamine and d-amphetamine (e.g., Baumann, *et al.*2012; Kehr *et al.* 2011; Hadlock *et al.*2011), methamphetamine and d-amphetamine are considered more prototypical dopamine releasers (Matsumoto et al.2014) and produce discriminative stimulus effects predominantly through dopaminergic mechanisms (for review see Berquist II and Fantegrossi 2018). In the current study, it is not surprising that methamphetamine produced slightly more substitution for the high and low training doses of mephedrone since methamphetamine does produce more serotonin release than d-amphetamine in addition to their dopamine and norepinephrine release (Kuczenki et al.1995; Matsumoto et al.2014). In a cross-substitution study, a dose of 2.0 mg/kg mephedrone fully substituted for 0.5 mg/kg d-amphetamine and the D₁ receptor antagonist, SCH39166, inhibited this substitution (Harvey et al.2017). This is good evidence that the component of mephedrone that substitutes for d-amphetamine has a significant component of D₁ receptor agonism. Doses of 2.5 and 5.0 mg/kg mephedrone fully substituted for both 10 mg/kg cocaine and 1.0 mg/kg methamphetamine training doses in drug discrimination assays (Gatch et al.2013). Therefore, when mephedrone is tested in rats trained to discriminate cocaine, d-amphetamine, and methamphetamine, full substitution of mephedrone for these cues occurs. However, reciprocal full cross-substitution is not always observed when the rats are trained to discriminate higher doses of mephedrone. For example, the drugs cocaine, d-amphetamine, and methamphetamine partially substituted between 40–80% for 3.0 or 3.2 mg/kg mephedrone (Berquist II et al.2017; Varner et al.2013) which was slightly higher substitution than in the current study. However, full substitution of methamphetamine, d-amphetamine, and cocaine for mephedrone was observed by lowering the training dose to 0.5 or 1.0 mg/kg mephedrone (current study; Berquist II et al.2017). These studies taken together continue to support the involvement of dopamine in the discriminative stimulus effects of mephedrone. When examined together, these substitution studies for methamphetamine, d-amphetamine, and cocaine support a key role for nonselective monoamine substrate/release and, to a slightly lesser extent, nonselective monoamine transporter inhibition in the discriminative stimulus effects of the low training dose mephedrone. The observation that cocaine, methamphetamine, and d-amphetamine produced less substitution in the higher training doses of mephedrone, suggests that dopamine and norepinephrine release and/or DAT and NET inhibition may be a minor component but not necessarily prominent part of these discriminative stimulus effects.

The underlying pharmacology of mephedrone and other transport inhibitor/substrates is challenging at best and drug discrimination is an excellent model to meet these challenges (for review see Berquist II and Fantegrossi 2018). The substitution experiments in the mephedrone trained rats from this study and others, indicates a prominent role for at least serotonin and dopamine release but it is difficult to tell if these processes and the effects on transporters are the basis of the discriminative cue or whether activation of a collection of serotonin and dopamine receptors post release are the basis of the discrimination. The best way to pharmacologically characterize or eliminate a particular receptor subtype from consideration is through the use of competitive antagonists. In the current study, multiple doses of four separate antagonists were used in an attempt to block the discriminative

stimulus and rate-decreasing effects of mephedrone. The primarily D_1 receptor antagonist, SCH23390, which blocked the discriminative stimulus effects of d-amphetamine and cocaine in previous studies (Callahan et al.1991; Schenk and Highgate 2018), produced significant rate-decreasing effects alone and in combination with mephedrone which made it basically impracticable to evaluate in the high training dose group with this antagonist. In the low training dose group, a dose of 0.025 mg/kg SCH23390 did block the discriminative stimulus effects of mephedrone in the 3/10 rats that were still responding. However, even in the low training dose group, the rates of responding were significantly low which compromised the conclusions on the role of D_1 receptors in the discriminative stimulus effects of mephedrone. The $D_{2/3}$ receptor antagonist sulpiride did not produce rate-decreasing effects that limited testing and actually produced rate-increasing effects by itself in the high training dose group and in combination with the low training dose of mephedrone. A dose of 2.0 mg/kg sulpiride produced a small antagonism of the training dose of 0.5 mg/kg mephedrone in the low training dose group, but a higher dose of 4.0 mg/kg failed to alter the discriminative stimulus effects. This non dose-dependent antagonism suggests a lack of competitive interactions between mephedrone and sulpiride, which may be important when considering the underlying mechanisms supporting the discriminative stimulus effects of mephedrone. The predominantly D_2 receptor antagonist haloperidol failed to block the discriminative stimulus effects of 3.2 mg/kg mephedrone (Varner et al.2013) but only the current study has examined D_1 or $D_{2/3}$ receptor antagonists in rats trained to discriminate mephedrone.

Based on the radioligand binding data for mephedrone and the full substitution of MDMA in both training groups, we predicated that selective 5-HT_{2C} receptor antagonist SB242084 and/or the 5-HT_{2A/2C} receptor antagonist ketanserin would block the discriminative stimulus effects of mephedrone. However, neither antagonist blocked the discriminative stimulus effects of the high training dose of mephedrone. SB242084 in combination with mephedrone produced high response rates in the lower training dose group but was also ineffective as an antagonist of the discriminative stimulus effects. In vitro, mephedrone was a 5-HT_{2A} receptor agonist in a calcium mobilization assay similar to MDMA (Rickli et al.2015a) but a 5-HT_{2A} receptor antagonist in a 5-HT-induced inositol monophosphate formation assay (Eshleman et al.2013). As ketanserin has highest affinity for 5-HT_{2A} receptors (Roth and Driscoll 2018), we tested ketanserin alone for substitution and as an antagonist of mephedrone. In the low training dose group, a small, insignificant shift to the right was observed after pretreatment with ketanserin and the testing of higher doses of ketanserin were limited by solubility. There was no evidence of substitution of either SB242082 or ketanserin in either training dose group eliminating the likelihood that 5-HT_{2C} or 5-HT_{2A} receptor antagonism is a component of the mephedrone discriminative stimulus. In total, the discriminative stimulus effects of mephedrone were basically insensitive to 5-HT_{2A} or 5-HT_{2C} receptor antagonists. It may be that a combination of antagonists are required to block mephedrone. Or, perhaps even if a component of the stimulus is block by one of these antagonists, this small antagonism is not enough to substantially change the discriminative stimulus effects of mephedrone and another potentially unstudied component of the mephedrone discriminative stimulus will preside enough to produce full mephedrone-like responding.

Acknowledgements

The authors wish to thanks Drs. Ellen Unterwald and Sara Jane Ward for their intellectual contributions and initial readings of this manuscript. Funding sources: R21DA032718, R01DA039139 and P30 DA013429–16

References

- Aarde SM, Angrish D, Barlow DJ, Wright MJ Jr, Vandewater SA, Creehan KM, et al.(2013) Mephedrone (4-methylmethcathinone) supports intravenous self-administration in Sprague-Dawley and Wistar rats. *Addict Biol.* 2013;18: 786–799.
- Baumann MH, Ayestas MA Jr, Partilla JS, Sink JR, Shulgin AT, Daley PF, et al.(2012) The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. *Neuropsychopharmacology.* 37: 1192–203. [PubMed: 22169943]
- Baumann MH, Walters HM, Niello M, Sitte HH. (2018) Neuropharmacology of Synthetic Cathinones. *Handb Exp Pharmacol.* Nov 8. doi: 10.1007/164_2018_178.
- Berquist MD II, Thompson NA, Baker LE (2017) Evaluation of training dose in male Sprague-Dawley rats trained to discriminate 4-methylmethcathinone. *Psychopharmacology (Berl)* 234 (21): 3271–3278. [PubMed: 28815279]
- Berquist MD, Fantegrossi WE (2018) Discriminative stimulus effects of psychostimulants. *Curr Top Behav Neurosci* 39:29–49. [PubMed: 28341944]
- Biezonski DK, Piper BJ, Shinday NM, Kim PJ, Ali SF, Meyer JS (2013) Effects of a short-course MDMA binge on dopamine transporter binding and on levels of dopamine and its metabolites in adult male rats. *Eur J Pharmacol* 701(1–3):176–80. [PubMed: 23276666]
- Bonano JS, Banks ML, Kolanos R, Sakloth F, Barnier ML, Glennon RA, Cozzi NV, Partilla JS, Baumann MH, Negus SS (2015) Quantitative structure-activity relationship analysis of the pharmacology of para-substituted methcathinone analogues. *Br J Pharmacol.* 172(10):2433–44. doi: 10.1111/bph.13030. [PubMed: 25438806]
- Callahan PM, Cunningham KA (1994) Involvement of 5-HT_{2C} receptors in mediating the discriminative stimulus properties of m-chlorophenylpiperazine (mCPP). *Eur J Pharmacol* 257(1–2): 27–38 [PubMed: 8082704]
- Callahan PM, Appel JB, Cunningham KA (1991) Dopamine D1 and D2 mediation of the discriminative stimulus properties of d-amphetamine and cocaine. *Psychopharmacology (Berl).* 103(1):50–5. [PubMed: 2006243]
- Creehan KM, Vandewater SA, Taffe MA (2015) Intravenous self-administration of mephedrone, methylone and MDMA in female rats. *Neuropharmacology* 92: 90–97. [PubMed: 25600245]
- DeLarge AF, Erwin LL, Winsauer PJ (2017) Atypical binding at dopamine and serotonin transporters contribute to the discriminative stimulus effects of mephedrone. *Neuropharmacology* 119: 62–75. [PubMed: 28396142]
- Eshleman AJ, Wolfrum KM, Hatfield MG, Johnson RA, Murphy KV, Janowsky A (2013) Substituted methcathinones differ in transporter and receptor interactions. *Biochem Pharmacol* 15: 85(12): 1803–1815. [PubMed: 23583454]
- Gannon BM, Fantegrossi WE (2016) Cocaine-like discriminative stimulus effects of mephedrone and naphyrone in mice. *J Drug Alcohol Res* 5: art236009. doi:10.4303/jdar/236009.
- Gatch MB, Taylor CM, Forster MJ (2013) Locomotor stimulant and discriminative stimulus effects of ‘bath salt’ cathinones. *Behav Pharmacol.* 24: 437–447. [PubMed: 23839026]
- Goodwin AK, Baker LE (2000) A three-choice discrimination procedure dissociates the discriminative stimulus effects of d-amphetamine and (+/-)-MDMA in rats. *Exp Clin Psychopharmacol* 8(3): 415–23. [PubMed: 10975633]
- Goodwin AK, Pynnonen DM, Baker LE (2003) Serotonergic-dopaminergic mediation of MDMA’s discriminative stimulus effects in a three-choice discrimination. *Pharmacol Biochem Behav* 74: 987–995. [PubMed: 12667914]
- Gommans J, Hijzen TH, Maes RA, Olivier B (1998) Discriminative stimulus properties of mCPP: evidence for a 5-HT_{2C} receptor mode of action. *Psychopharmacology (Berl).* 137(3):292–302. [PubMed: 9683007]

- Gregg RA, Baumann MH, Partilla JS, Bonano JS, Vouga A, Tallarida CS, Velvadapu V, Smith GR, Peet MM, Reitz AB, Negus SS, Rawls SM (2015) Stereochemistry of mephedrone neuropharmacology: enantiomer-specific behavioural and neurochemical effects in rats. *Brit J Pharmacol* 72(3): 883–94.
- Hadlock GC, Webb KM, McFadden LM, Chu PW, Ellis JD, Allen SC, et al. (2011) 4-Methylmethcathinone (mephedrone): neuropharmacological effects of a designer stimulant of abuse. *J Pharmacol Exp Ther* 339: 530–536. [PubMed: 21810934]
- Harvey EL, Burroughs RL, Baker LE (2017) Effects of D1 and D2 receptor antagonists on the discriminative stimulus effects of methylendioxypropylvalerone and mephedrone in male Sprague-Dawley rats trained to discriminate D-amphetamine. *Behav Pharmacol* 28: 586–589. [PubMed: 28704276]
- Harvey EL, Baker LE (2016) Differential effects of 3,4-methylenedioxypropylvalerone (MDPV) and 4-methylmethcathinone (mephedrone) in rats trained to discriminate MDMA or a d-amphetamine + MDMA mixture. *Psychopharmacology (Berl)* 233: 673–680. [PubMed: 26558618]
- Kehr J, Ichinose F, Yoshitake S, Gojny M, Sievertsson T, Nyberg F, Yoshitake T (2011) Mephedrone, compared with MDMA (Ecstasy) and amphetamine, rapidly increases both dopamine and 5-HT levels in nucleus accumbens of awake rats. *Br J Pharmacol* 164: 1949–1958. [PubMed: 21615721]
- Kuczenski R, Segal DS, Cho AK, Melga W (1995) Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to stereoisomers of amphetamine and methamphetamine. *J Neurosci* 15:1308–1317. [PubMed: 7869099]
- Lisek R, Xu W, Yuvashova E, Chiu YT, Reitz AB, Liu-Chen LY, Rawls SM (2012) Mephedrone ('bath salt') elicits conditioned place preference and dopamine-sensitive motor activation. *Drug Alcohol Depend* 126: 257–262. [PubMed: 22652295]
- Luethi D, Kolaczynska KE, Docci L, Krahenbuho S, Hoener MC, Liechti ME (2018) Pharmacological profile of mephedrone analogs and related new psychoactive substances. *Neuropharmacology* 134: 4–12. [PubMed: 28755886]
- Martinez-Clemente J, Escubedo E, Pubill D, Camarasa J (2012) Interaction of mephedrone with dopamine and serotonin targets in rats. *Eur Neuropsychopharmacol* 22: 231–236. [PubMed: 21824752]
- Marusich JA, Lefever TW, Novak SP, Blough BE, Wiley JL (2013) Prediction and prevention of prescription drug abuse: role of preclinical assessment of substance abuse liability. *Methods Rep RTI Press* 1–14. doi:10.3768/rtipress.2013.op.0014.1307.
- Matsumoto T, Maeno Y, Kato H, Seko-Nakamura Y, Monma-Ohtaki J, Ishiba A (2014) 5-hydroxytryptamine- and dopamine-releasing effects of ring-substituted amphetamines on rat brain: a comparative study using in vivo microdialysis. *Eur Neuropsychopharmacol* 24: 1362–1370. [PubMed: 24862256]
- Pettibone DJ, Williams M (1984) Serotonin-releasing effects of substituted piperazines in vitro. *Biochem Pharmacol* 33 (9): 1531–5. [PubMed: 6610423]
- Philogene-Khalid HL, Simmons SJ, Nayak S, Martorana RM, Su SH, Caro Y, Ranieri B, DiFurio K, Mo L, Gentile TA, Murad A, Reitz AB, Muschamp JW, Rawls SM (2017a). Stereoselective differences between the reinforcing and motivational effects of cathinone-derived 4-methylmethcathinone (Mephedrone) in self-administering rats. *ACS Chem Neurosci* 8(12):2648–2654. doi: 10.1021/acchemneuro.7b00212. [PubMed: 28885007]
- Philogene HL, Smith GR, Reitz AB, Liu-Chen L, Rawls SM (2017b) S enantiomer of the synthetic cathinone mephedrone reduces anxiety- and depressant-like effects caused by cocaine or MDPV withdrawal in rats. *Drug Alcohol Depend*. 178:119–125. doi: 10.1016/j.drugalcdep.2017.04.024. [PubMed: 28646714]
- Pigott A, Frescas S, McCorvy JD, Huang XP, Roth BL, Nichols DE (2012) trans-2-(2,5-Dimethoxy-4-iodophenyl)cyclopropylamine and trans-2-(2,5-dimethoxy-4-bromophenyl)cyclopropylamine as potent agonists for the 5-HT(2) receptor family. *Beilstein J Org Chem* 1705–9. doi: 10.3762/bjoc.8.194. [PubMed: 23209503]
- Rickli A, Hoener MC, Liechti ME (2015a) Monoamine transporter and receptor interaction profiles of novel psychoactive substances: Para-halogenated amphetamines and pyrovalerone cathinones. *Eur Neuropsychopharmacology* 25: 365–376.

- Rickli A, Luethi D, Reinisch J, Buchy D, Hoener MC, Liechti ME (2015b) Receptor interaction profiles of novel N-2-methoxybenzyl (NBOMe) derivatives of 2,5-dimethoxy-substituted phenethylamines (2C drugs). *Neuropharmacology* 99: 546–553. [PubMed: 26318099]
- Roth BL; Driscoll J (2018) PDSP Ki Database". Psychoactive Drug Screening Program (PDSP). University of North Carolina at Chapel Hill and the United States National Institute of Mental Health. Retrieved 26 November 2018.
- Schenk S and Highgate Q (2018) Dopamine and serotonin antagonists fail to alter the discriminative stimulus properties of $\pm$ methylenedioxymethamphetamine. *Behav Pharmacol.* 2018 Sep 7. doi: 10.1097/FBP.0000000000000442. [Epub ahead of print]
- Simmler LD, Rickli A, Schramm Y, Hoener MC, Liechti ME (2014b) Pharmacological profiles of aminoindanes, piperazines, and pipradrol derivatives. *Biochem Pharmacol* 88:237–244. [PubMed: 24486525]
- Simmler LD, Buser TA, Donzelli M, Schramm Y, Dieu L-H, Huwyler J, Chaboz S, Hoener MC, Liechti ME (2013) Pharmacological characterization of designer cathinones in vitro. *Br J Pharmacol* 168: 458–470. [PubMed: 22897747]
- Smith DA, Negus SS, Poklis JL, Blough BE, Banks ML (2016) Cocaine-like discriminative stimulus effects of alpha-pyrrolidinovalerophenone, methcathinone and their 3,4-methylenedioxy or 4-methyl analogs in rhesus monkeys. *Addict Biol* doi: 10.1111/adb.12399.
- Suyama JA, Sakloth F, Kolanos R, Glennon RA, Lazenka MF, Negus SS, Banks ML (2016) Abuse-related neurochemical effects of para-substituted methcathinone analogs in rats: Microdialysis studies of nucleus accumbens dopamine and serotonin. *J Pharmacol Exp Ther* 36: 182–190.
- Varner KJ, Daigle K, Weed PF, Lewis PB, Mahne SE, Sankaranarayanan A, et al.(2013) Comparison of the behavioral and cardiovascular effects of mephedrone with other drugs of abuse in rats. *Psychopharmacology (Berl)* 225: 675–685. [PubMed: 22972412]
- Zaami S, Giorgetti R, Pichini S, Pantano F, Marinelli E, Busardo FP (2018) Synthetic cathinones related fatalities: an update. *Eur Rev Med Pharmacol Sci.* 22(1):268–274. doi: 10.26355/eurrev20180114129. [PubMed: 29364498]
- Zea-Ponce Y, Kegeles LS, Guo N, Raskin L, Bakthavachalam V, Laruelle M (2002) Pharmacokinetics and brain distribution in non-human primate of R(-)[123I]DOI, A 5-HT(2A/2C) serotonin agonist. *Nucl. Med. Biol* 29, 575e583. [PubMed: 12088728]

Figure 1.

Dose-response curves for mephedrone and morphine in rats trained to discriminate either 3.2 mg/kg (left panels) or 0.5 mg/kg (right panels) mephedrone from saline and R-mephedrone and S-mephedrone in rats trained to discriminate 0.5 mg/kg mephedrone (right panels).

Abscissae: doses of drugs, in mg/kg. Points above S indicate saline tests. *Ordinate:* the percentage of the total responses made on the mephedrone-appropriate lever (upper panels) or the response rate measured as total responses made on both levers divided by the total time in seconds (lower panels). The dashed lines in the upper panels represent no substitution (0–20%), partial substitution (20–80%) and full substitution (80–100%). The dashed lines in the lower panels represent the saline response rates during the saline test session for each training group. The numbers of rats represented in the discrimination panels/number of rats tested for each dose of drug: *High Training Dose* - [Saline (10/10); mephedrone (0.05 mg/kg-10/10, 0.15 mg/kg-10/10, 0.5 mg/kg-10/10, 1.6 mg/kg-8/9, 3.2 mg/kg-11/11, 5.0 mg/kg-5/8); morphine (0.28 mg/kg-8/8, 0.5 mg/kg-6/7, 5.0 mg/kg-1/6)]; *Low Training Dose* - [Saline (22/22); mephedrone (0.05 mg/kg-23/23, 0.15 mg/kg-23/23, 0.5 mg/kg-22/22, 1.6 mg/kg-22/23); morphine (0.28 mg/kg-12/12, 0.5 mg/kg-12/12, 5.0 mg/kg-11/11); S-mephedrone (0.05 mg/kg-12/12, 0.15 mg/kg-12/12, 0.5 mg/kg-12/12); R-mephedrone (0.5 mg/kg-12/12, 1.6 mg/kg-12/12)]. *, significantly different from saline test response rates at least $p < 0.05$ as determined by Dunnett Multiple Comparisons post hoc test. Vertical lines represent $\pm$ S.E.M.

Figure 2.

Cocaine, d-amphetamine, and methamphetamine substitution tests in rats trained to discriminate 3.2 mg/kg mephedrone (left panels) or 0.5 mg/kg mephedrone (right panels). The numbers of rats represented in the discrimination panels/number of rats tested for each dose of drug: *High Training Dose* - [Cocaine (0.5 mg/kg-8/8, 0.9 mg/kg-8/8, 5.0 mg/kg-8/8, 10 mg/kg-8/8, 15 mg/kg-6/6); d-amphetamine (0.03125 mg/kg-6/6, 0.0625 mg/kg-7/7, 0.125 mg/kg-6/6, 0.25 mg/kg-6/6, 0.5 mg/kg-6/6, 1.0 mg/kg-4/6, 2.0 mg/kg-2/7); methamphetamine (0.15 mg/kg-8/8, 0.28 mg/kg-8/8, 0.5 mg/kg-8/8, 1.0 mg/kg-6/7, 3.0 mg/kg-3/6)]; *Low Training Dose* - [Cocaine (0.5 mg/kg-12/12, 0.9 mg/kg-12/12, 5.0 mg/kg-12/12, 10 mg/kg-8/8, 15 mg/kg-7/8); d-amphetamine (0.03125 mg/kg-10/10, 0.0625 mg/kg-10/10, 0.125 mg/kg-8/8, 0.25 mg/kg-9/9, 0.5 mg/kg-17/17, 1.0 mg/kg-6/8, 2.0 mg/kg-6/8); methamphetamine (0.15 mg/kg-20/20, 0.28 mg/kg-18/18, 0.5 mg/kg-20/20, 1.0 mg/kg-8/8, 3.0 mg/kg-4/8)]. Other details as in Figure 1.

Figure 3.

DOI, MDMA, WAY163909, and mCPP substitution tests in rats trained to discriminate 3.2 mg/kg mephedrone (left panels) or 0.5 mg/kg mephedrone (right panels). The numbers of rats represented in the discrimination panels/number of rats tested for each dose of drug: *High Training Dose* - [DOI (0.1 mg/kg-8/8, 0.5 mg/kg-2/6, 1.0 mg/kg-3/6, 2.0 mg/kg-3/6); MDMA (0.5 mg/kg-7/7, 1.6 mg/kg-8/8, 5.0 mg/kg-8/8, 9.0 mg/kg-5/7); WAY163909 (0.05 mg/kg-6/6, 0.15 mg/kg-6/6, 0.5 mg/kg-5/6, 0.75 mg/kg-5/6, 1.0 mg/kg-2/6)]; *Low Training Dose* - [DOI (0.1 mg/kg-9/9, 0.5 mg/kg-5/9, 1.0 mg/kg-2/8, 2.0 mg/kg-1/8); MDMA (0.5 mg/kg-12/12, 1.6 mg/kg-12/12, 5.0 mg/kg-10/11, 9.0 mg/kg-6/12); WAY163909 (0.05 mg/kg-8/8, 0.15 mg/kg-7/8, 0.5 mg/kg-7/9, 0.75 mg/kg-5/9, 1.0 mg/kg-3/10); mCPP (0.3 mg/kg-12/12, 0.5 mg/kg-12/12, 1.6 mg/kg-9/12)]. Other details as in Figure 1.

Figure 4.

Effects of D_1 receptor antagonist SCH23390 in rats trained to discriminate 3.2 mg/kg mephedrone (left panels) or 0.5 mg/kg mephedrone (right panels). *Abscissae*: doses of mephedrone, in mg/kg. Points above A indicate doses of SCH23390 administered alone. Dashed lines connecting the data points in the upper, left panel indicate that the discrimination data are obtained from less than half the rats tested. The numbers of rats represented in the discrimination panels/number of rats tested for each dose of drug: *High Training Dose* - [Saline-(10/10); mephedrone (0.05 mg/kg-10/10, 0.15 mg/kg-10/10, 0.5 mg/kg-10/10, 1.6 mg/kg-8/10, 3.2 mg/kg-10/10, 5.0 mg/kg-5/8; 0.0125 mg/kg SCH23390 alone (3/10) and with mephedrone doses (0.05 mg/kg-0/6, 0.15 mg/kg-0/6, 0.5 mg/kg-0/7, 1.6 mg/kg-3/6, 3.2 mg/kg-0/6); 0.025 mg/kg SCH23390 alone (3/8) and with mephedrone doses (0.05 mg/kg-0/6, 0.15 mg/kg-0/6, 0.5 mg/kg-0/6, 1.6 mg/kg-2/6, 3.2 mg/kg-3/6); 0.06 mg/kg SCH23390 alone (0/9) and with mephedrone doses (0.05 mg/kg-0/6, 0.15 mg/kg-0/6, 0.5 mg/kg-0/6, 1.6 mg/kg-0/6, 3.2 mg/kg-1/6)]. *Low Training Dose* - [Saline-(21/21); mephedrone (0.05 mg/kg-20/20, 0.15 mg/kg-20/20, 0.5 mg/kg-19/19, 1.6 mg/kg-19/20); 0.0125 mg/kg SCH23390 alone (18/18) and with mephedrone doses (0.05 mg/kg-4/10, 0.15 mg/kg-3/9, 0.5 mg/kg-9/10, 1.6 mg/kg-7/8); 0.025 mg/kg SCH23390 alone (15/18) and with mephedrone doses (0.05 mg/kg-1/9, 0.15 mg/kg-1/9, 0.5 mg/kg-3/10, 1.6 mg/kg-4/8); 0.06 mg/kg SCH23390 alone (5/20) and with mephedrone doses (0.05 mg/kg-0/7, 0.15 mg/kg-1/6, 0.5 mg/kg-1/11, 1.6 mg/kg-1/7)]. *, significantly different from saline test response rates at least $p < 0.05$ or ^, significantly different from mephedrone training dose values at least $p < 0.05$ as determined by Dunnett Multiple Comparisons post hoc tests. Other details as in Figure 1.

Figure 5-

Effects of $D_{2/3}$ receptor antagonist sulpiride in rats trained to discriminate 3.2 mg/kg mephedrone (left panels) or 0.5 mg/kg mephedrone (right panels). Points above A indicate doses of sulpiride administered alone. The numbers of rats represented in the discrimination panels/number of rats tested for each dose of drug: *High Training Dose* - [Saline-(10/10); mephedrone (0.05 mg/kg-10/10, 0.15 mg/kg-10/10, 0.5 mg/kg-10/10, 1.6 mg/kg-8/10, 3.2 mg/kg-10/10, 5.0 mg/kg-5/8); sulpiride 2.0 mg/kg alone (9/9) and with mephedrone doses (0.05 mg/kg-6/6, 0.15 mg/kg-6/6, 0.5 mg/kg-7/7, 1.6 mg/kg-6/6, 3.2 mg/kg-6/7); sulpiride 4.0 mg/kg alone (8/8) and with mephedrone doses (0.05 mg/kg-6/6, 0.15 mg/kg-6/6, 0.5 mg/kg-6/6, 1.6 mg/kg-6/6, 3.2 mg/kg-6/7)]. *Low Training Dose* - [Saline-(21/21); mephedrone (0.05 mg/kg-20/20, 0.15 mg/kg-20/20, 0.5 mg/kg-19/19, 1.6 mg/kg-19/20); sulpiride 2.0 mg/kg alone (17/17) and with mephedrone doses (0.05 mg/kg-9/9, 0.15 mg/kg-8/8, 0.5 mg/kg-10/10, 1.6 mg/kg-11/11); sulpiride 4.0 mg/kg alone (12/12) and with mephedrone doses (0.05 mg/kg-7/7, 0.15 mg/kg-8/8, 0.5 mg/kg-7/7, 1.6 mg/kg-5/8)]. *, significantly different from saline test response rates at least $p < 0.05$ or ^, significantly different from mephedrone training dose values at least $p < 0.05$ as determined by Dunnett Multiple Comparisons post hoc tests. Other details as in Figure 1.

Figure 6.

Effects of 5-HT_{2C} receptor antagonist SB242084 in rats trained to discriminate 3.2 mg/kg mephedrone (left panels) or 0.5 mg/kg mephedrone (right panels). Points above A indicate doses of SB242084 administered alone. The numbers of rats represented in the discrimination panels/number of rats tested for each dose of drug: *High Training Dose* - [Saline-10/10; mephedrone (0.05 mg/kg-10/10, 0.15 mg/kg-10/10, 0.5 mg/kg-10/10, 1.6 mg/kg-8/10, 3.2 mg/kg-10/10, 5.0 mg/kg-5/8); SB242084 0.5 mg/kg alone (8/8) and with mephedrone doses (0.05 mg/kg-6/6, 0.15 mg/kg-5/6, 0.5 mg/kg-6/6, 1.6 mg/kg-6/6, 3.2 mg/kg-6/6); SB242084 1.0 mg/kg alone (8/8) and with mephedrone doses (0.05 mg/kg-6/6, 0.15 mg/kg-7/7, 0.5 mg/kg-6/6, 1.6 mg/kg-7/8, 3.2 mg/kg-6/6)]. *Low Training Dose* - [Saline (21/21; mephedrone (0.05 mg/kg-20/20, 0.15 mg/kg-20/20, 0.5 mg/kg-19/19, 1.6 mg/kg-19/20); SB242084 0.5 mg/kg alone (16/16) and with mephedrone doses (0.05 mg/kg-9/9, 0.15 mg/kg-9/9, 0.5 mg/kg-8/8, 1.6 mg/kg-7/7); SB242084 1.0 mg/kg alone (14/14) and with mephedrone doses (0.05 mg/kg-10/10, 0.15 mg/kg-8/8, 0.5 mg/kg-10/10, 1.6 mg/kg-11/11)]. *, significantly different from saline test response rates at least $p < 0.05$ or ^, significantly different from mephedrone training dose values at least $p < 0.05$ as determined by Dunnett Multiple Comparisons post hoc tests. Other details as in Figure 1.

Figure 7.

Effects of 5-HT_{2A/2C} receptor antagonist ketanserin in rats trained to discriminate 3.2 mg/kg mephedrone (left panels) or 0.5 mg/kg mephedrone (right panels). Points above A indicate doses of ketanserin administered alone. The numbers of rats represented in the discrimination panels/number of rats tested for each dose of drug: *High Training Dose*- [Saline-9/9; mephedrone (0.05 mg/kg-9/9, 0.15 mg/kg-9/9, 0.5 mg/kg-9/9, 1.6 mg/kg-7/9, 3.2 mg/kg-9/9, 5.0 mg/kg-4/7); ketanserin 1.0 mg/kg alone (9/9) and with mephedrone doses (0.05 mg/kg-6/6, 0.15 mg/kg-7/7, 0.5 mg/kg-6/6, 1.6 mg/kg-6/6, 3.2 mg/kg-6/7); ketanserin 1.5 mg/kg alone (8/8) and with mephedrone doses (0.05 mg/kg-6/6, 0.15 mg/kg-7/7, 0.5 mg/kg-6/6, 1.6 mg/kg-6/6, 3.2 mg/kg-6/6)]; *Low Training Dose* - [Saline -12/12; mephedrone (0.05 mg/kg-11/11, 0.15 mg/kg-11/11, 0.5 mg/kg-10/10, 1.6 mg/kg-10/11); ketanserin 1.0 mg/kg alone (12/12) and with mephedrone doses (0.05 mg/kg-8/8, 0.15 mg/kg-8/8, 0.5 mg/kg-9/9, 1.6 mg/kg-4/8)]. *, significantly different from saline test response rates at least $p < 0.05$ or ^, significantly different from mephedrone training dose values at least $p < 0.05$ as determined by Dunnett Multiple Comparisons post hoc tests. Other details as in Figure 1.