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Contribution of Monoaminergic Mechanisms to the Discriminative Stimulus Effects of 3,4-Methylenedioxypyrovalerone (MDPV) in Sprague-Dawley Rats

Risca

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by

Harmony I. Risca

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Thesis Committee:

Lisa E. Baker, Ph.D., Chair
Cynthia J. Pietras, Ph.D.
Ron Van Houten, Ph.D.

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Harmony I. Risca, M.A.

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3,4-Methylenedioxypyrovalerone (MDPV) is a popular synthetic cathinone reported to have a high abuse potential and comparable pharmacological actions to those of cocaine. The aim of this study was to evaluate a variety of monoaminergic agents for substitution, potentiation, or antagonism in rats trained to discriminate MDPV. Male Sprague-Dawley rats were trained to discriminate 0.5 mg/kg MDPV and a variety of monoaminergic drugs were tested for substitution and/or potentiation of the MDPV cue. In separate experiments, stimulus antagonism tests were conducted with selected dopamine antagonists or serotonin antagonists in rats trained to discriminate 1 mg/kg MDPV. Full substitution for MDPV was observed with cocaine, (±)-MDMA, (+)-MDMA and (±)-MDA; (+)-MDA produced significant partial substitution, whereas (-)-MDMA or (-)-MDA did not substitute. Although neither GBR 12909 nor desipramine substituted for MDPV, these substances potentiated MDPV discrimination. Sch 23390 and haloperidol both dose-dependently attenuated MDPV discrimination, whereas none of the 5-HT antagonists tested altered MDPV discrimination. These findings indicate MDPV's interoceptive stimulus effects are mediated predominantly by dopaminergic actions, although serotonergic and/or noradrenergic modulation of these effects cannot be ruled out. Further investigations into the precise neurochemical actions responsible for MDPV discrimination may serve to inform medication discovery and development for the treatment of MDPV abuse.

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CHAPTER 1

INTRODUCTION

Synthetic Cathinones

Substance abuse and addiction have long been a public health concern, but the recent popularity in the marketing of synthetic alternatives as "legal highs" has become a worldwide health concern. One of the three most common classes of "new psychoactive substances" includes the synthetic cathinones ("bath salts") (Rosenbaum & Carreiro, 2012). Although most synthetic cathinones used today are manufactured by foreign laboratories and sold via the internet, cathinone is a naturally occurring psychoactive substance that can be found in the leaves of the khat (*Catha edulis*) plant (Baumann et al., 2017; Valente et al., 2014). Khat leaves and twigs have been chewed for centuries in East African countries for their mild amphetamine-like euphoric effects long before synthetic cathinones emerged in recreational drug markets (Valente et al., 2014).

The initial popularity of synthetic cathinone use arose in response to attempts to elude legal restrictions on other popular drugs of abuse, such as cocaine and 3,4-methylenedioxymethamphetamine (MDMA) (Valente et al., 2014). Although synthetic cathinones are now listed as schedule I controlled substances in the United States, illicit use remains popular among recreational users. Emergency room visits and poison control reports related to synthetic cathinones (U.S. Department of Justice National Drug Intelligence Center (NDIC), 2011) have recently raised public health awareness regarding these substances.

3,4-Methylenedioxypyrovalerone (MDPV) is one of several synthetic cathinone derivatives and a popular constituent of illicit "bath salts" commonly associated with emergency department reports related to "bath salt" abuse (Centers for Disease Control and Prevention

(CDC), 2011; Froberg et al., 2015; NDIC, 2011; Spiller et al., 2011). Physiological effects of MDPV toxicity include tachycardia, hypertension, arrhythmias, hyperthermia, sweating, rhabdomyolysis, seizures, cerebral edema, cardiorespiratory collapse, myocardial infarction, and death. In addition to life-threatening physiological symptoms, reports of behavioral effects including panic attacks, anxiety, agitation, severe paranoia, hallucinations, psychosis, suicidal ideation, self- mutilation, and aggression have been noted (CDC, 2011; Ross et al., 2012).

Neuropharmacology of MDPV

Previous research indicates MDPV has neuropharmacological and behavioral properties comparable to those of cocaine, a highly addictive central nervous system stimulant derived from the leaves of the coca plant, and the psychedelic-stimulant and popular club drug MDMA, "Ecstasy" (Aarde et al., 2015; Cameron et al., 2013; Fantegrossi et al., 2013; Gatch et al., 2013; Ross et al., 2012). Studies using established drug discovery techniques, such as *in vitro* receptor binding, have revealed that MDPV potently blocks dopamine and norepinephrine uptake, with relatively weaker effects on serotonin uptake (Simmler et al., 2013; Eshleman et al., 2013). Compared to cocaine, MDPV is up to 50-fold more potent at the dopamine transporter (DAT), approximately 10-fold more potent at the norepinephrine transporter (NET), and 10-fold less potent at the serotonin transporter (Baumann et al., 2013). This enhanced potency may be responsible for the severity of physiological and behavioral reports associated with MDPV toxicity (Froberg et al., 2015; Spiller et al., 2011).

Psychopharmacology of MDPV

The abuse liability of MDPV has been confirmed by several preclinical reports that this substance establishes conditioned place preference (King et al., 2015a; 2015b) and maintains self-administration in rodents (Schindler et al., 2016; Aarde et al., 2013; Aarde et al., 2015;

Watterson et al., 2014). A recent report indicates MDPV is approximately 10-fold more potent and approximately three-fold more effective at maintaining responding under a progressive ratio schedule compared to cocaine (Gannon et al., 2017). Determining the precise neurochemical actions responsible for MDPV's abuse liability is critical to the discovery and development of treatment medications.

Drug Discrimination Assay

Drug discrimination is a widely accepted *in vivo* preclinical assay used to evaluate the neurochemical substrates underlying the interoceptive stimulus effects of drugs (Baker, 2017). Four basic components are typically involved in drug discrimination paradigms: '1) the subject and their "motivational condition"; 2) drug dose that exerts an effect on the subject, or its vehicle, and precedes a response by the subject; 3) an appropriate (or correct) response; and 4) presentation of reinforcement' (Glennon & Young, 2011, p. 8). A drug is established as a discriminative stimulus when drug administration consistently precedes reinforcement of a particular response (e.g., right lever press) and never precedes reinforcement of an alternative response (e.g., left lever press). Subjects are most typically trained to discriminate the centrally-mediated effects of a drug from vehicle (Stolerman et al., 1999). Such training and methodology allows for both qualitative and quantitative data to be collected, alluding to the stimulus properties of a drug (Glennon & Young, 2011).

Drug Discrimination Studies with MDPV

Despite the predictive utility of the drug discrimination paradigm, few published studies have employed such methods to train animals to discriminate MDPV. These studies have noted similar interoceptive stimulus effects between MDPV and several established psychoactive stimulants, such as methamphetamine, d-amphetamine, cocaine, and MDMA (Fantegrossi et al.,

2013; Berquist & Baker, 2017). Some discrepancies between these studies are worth noting, particularly with regard to stimulus substitution with MDMA. Fantegrossi reported full substitution with MDMA in mice trained to discriminate 0.3 mg/kg MDPV, whereas Berquist and Baker (2017) found that MDMA failed to fully substitute in rats trained to discriminate 0.3 mg/kg MDPV. Other methodological differences besides species (e.g., reinforcer type and reinforcement schedule) were noted between these studies and could contribute to the inconsistent findings.

Several other studies have evaluated MDPV for substitution in animals trained to discriminate other stimulants. Full substitution was observed with MDPV in rats trained to discriminate d-amphetamine (Harvey et al., 2017) or cocaine (Gatch et al., 2013; Gannon et al., 2016), although only partial substitution was observed with MDPV in rats trained to discriminate MDMA (Harvey & Baker, 2016).

Complex Interoceptive Stimulus Studies of MDMA

The equivocal findings regarding substitution between MDPV and MDMA may be related to MDMA's complex cues involving serotonergic and dopaminergic actions that may be dissociable and dependent on discrimination training methods (Baker et al., 1995; Goodwin & Baker, 2000; Goodwin et al. 2003). Previous studies utilizing drug discrimination procedures to assess the stereoisomers of MDMA and 3,4-Methylenedioxyamphetamine (MDA) suggest that the discriminative stimulus effects of (+)-MDA are more similar to those of amphetamine, whereas the discriminative stimulus effects of (-)-MDA, and (-)-MDMA are more comparable to those of LSD (Baker et al., 1995; Broadbent et al., 1992; Callahan & Appel, 1988; Glennon & Young, 1984).

Research Objective

In an effort to evaluate the neurochemical actions underlying the discriminative stimulus effects of MDPV the present study conducted two separate experiments (Experiment 1 and Experiment 2) using two different doses of MDPV (0.5 and 1.0 mg/kg) previously studied in our lab. Experiment 1 consisted of stimulus substitution with MDMA, MDA, and their optical isomers, along with monoamine reuptake inhibitors with varying selectivity for DAT, NET, or SERT in rats trained to discriminate 0.5 mg/kg MDPV to aid in the classification of MDPV's interoceptive effects. Additionally, Experiment 2 assessed dopamine and serotonin antagonists for attenuation of the MDPV cue in rats trained to discriminate 1.0 mg/kg MDPV.

CHAPTER II

METHODS

Subjects

Fourteen male Sprague-Dawley rats (Experiment 1: n=7; Experiment 2: n=7) (Charles River Laboratories, Wilmington, MA, USA) were housed individually in polycarbonate cages with corncob bedding (Harlan Laboratories, Haslett, MI, USA) in animal facilities maintained at constant 20 ± 2°C, with a humidity of 50 ± 5% under a 12:12 light/dark cycle (lights on from 07:00 to 19:00h). Animals were provided water *ad libitum* in home cages and fed restricted diets of commercial rodent chow (Purina®, Richmond, IN, USA) to maintain 85-90% of free-feeding weights (340-440g). 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).

Apparatus

Training and testing procedures were conducted in seven standard operant conditioning chambers equipped with three retractable levers, a food pellet dispenser and fan on the front panel, a 28-V house light on the back panel, and housed within sound-attenuating shells (ENV-001; Med Associates Inc., St. Albans, Vermont, USA). Experimental events were controlled using Med-PC IV software (version IV; Med Associates Inc.). Dustless Precision Pellets (45 mg; Product# F0021; BioServ, Flemington, NJ) were used as reinforcements for lever pressing.

Drugs

Cocaine-hydrochloride, 3,4-methylenedioxypyrovalerone-hydrochloride (MDPV), (±)-3,4-methylenedioxymethamphetamine-hydrochloride (MDMA), (±)-3,4-

methylenedioxyamphetamine-hydrochloride (MDA), and the optical isomers of MDMA and MDA were provided by the National Institute on Drug Abuse Drug Control Supply Program (Bethesda, MD). GBR 12909 bismethanesulfonate monohydrate was prepared in the Chemical Biology Research Branch, National Institute on Drug Abuse and the National Institute on Alcohol Abuse and Alcoholism. MDL 100,907, and WAY 100,635 were provided by the National Institutes of Mental Health Chemical Synthesis and Drug Supply Program. Pirenperone was purchased from Santa Cruz Biotechnology (Mississauga, ON). Desipramine-hydrochloride, (+)-Sch 23390-hydrochloride, and haloperidol were purchased from Sigma-Aldrich ® (St. Louis, MO). Haloperidol was dissolved in a few drops of 0.1 M HCl, diluted in sterile water, and the pH was adjusted as needed with 0.1 M NaOH. Pirenperone was dissolved in 30% cyclodextrin (2-hydroxypropyl-beta-cyclodextrin) at a concentration of 1 mg/ml and then 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. All drugs were administered by intraperitoneal (i.p.) injection at a volume of 1 ml/kg. The range of doses and pre-session injection intervals for MDPV and all other compounds tested were selected based on previous studies conducted in our laboratory.

Preliminary Training

Preliminary and discrimination training procedures were identical in *Experiment 1* and *Experiment 2*, apart from training dose, and were similar to previous studies previously performed in the present lab (Berquist & Baker, 2017; Harvey & Baker, 2016). Initial training involved two 60-min sessions, one per day for two consecutive days in which no levers were presented and food pellets were delivered under a fixed-time 60 second (FT60") schedule to acclimated rats to the operant chambers and familiarize them towards the pellet dispenser.

Subsequent training sessions lasted 20 minutes per day and were conducted 6-7 days a week. To reduce the likelihood of lever bias, center levers were exclusively used during initial lever training. Reinforcement was delivered under a fixed-ratio (FR) schedule that was gradually incremented from FR 1 to FR 20. Once rats were reliably pressing the center lever on the FR 20 schedule, errorless training sessions began. During errorless training, rats were injected via i.p. with either vehicle or drug prior to session depending on whether the left or right lever was extended. Lever assignment to stimulus condition was counterbalanced among rats in each experiment. Errorless sessions were conducted in the following order: V, V, D, D, V, D, V, V, D, D, V, D. Discrimination training began once subjects were responding reliably on a FR 20 schedule on both the drug and vehicle-paired levers.

Discrimination Training

Discrimination training involved the simultaneous presentation of both levers in which rats were trained to discriminate either 0.5 mg/kg MDPV (Experiment 1) or 1.0 mg/kg MDPV (Experiment 2) from vehicle under a FR 20 schedule of food reinforcement. Drug and vehicle training sessions alternated with the stipulation that the same stimulus condition occurred no more than twice consecutively (e.g., D, D, V, D, V, V), but in no particular order. The performance criteria for stimulus control was a minimum of eight out of ten consecutive discrimination training sessions with 80% or higher correct lever responses prior to delivery of the first reinforcer and for the total session.

After stimulus control was established, test sessions were conducted as described below for each experiment. Test sessions were similar to training sessions, with the exception that responses were not reinforced and sessions ended upon completion of the first FR 20 or after 20 minutes, whichever occurred first. Testing criteria between sessions required subjects to

complete at least one drug and at least one vehicle training session consecutively with 80% or higher injection-appropriate responding.

Experiment 1

Stimulus Substitution and Potentiation Tests

In rats trained to discriminate 0.5 mg/kg MDPV, the following drugs (doses, pre-injection interval) were assessed for substitution: MDPV (0.05, 0.1, 0.5 mg/kg, 15 min), (±)-MDA, (±)-MDMA, (+)-MDA, (-)-MDA, (+)-MDMA, (-)-MDMA (0.75, 1.5, 3.0 mg/kg, 15 min), GBR 12909 (5, 10, 20, 40 mg/kg, 30 min), cocaine (2.5, 5, 10, 20 mg/kg, 15 min), and desipramine (0.0, 3.2, 5.6, 10 mg/kg, 30 min). Additionally, Sch 23390 (0.01, 0.03, 0.1 mg/kg, 30 min) was assessed for antagonism of 0.5 mg/kg MDPV. Finally, desipramine (3.2 mg/kg, 30 min), GBR 12909 (40 mg/kg, 30 min), or vehicle were administered as a pretreatment to each MDPV dose (0.05, 0.1, 0.5 mg/kg, 15 min) to assess potentiation of MDPV discrimination.

Experiment 2

Stimulus Antagonism Tests

After determination of the dose-response curve with MDPV (0.1, 0.3, 1.0 mg/kg, 15 min), the following drugs were assessed for antagonism of 1.0 mg/kg MDPV: Sch 23990 (0.01, 0.03, 0.1, 0.3 mg/kg, 30 min), haloperidol (0.125, 0.25, 0.5 mg/kg, 60 min), pirenperone (0.16, 0.32, 0.64 mg/kg, 60 min), MDL 100,907 (0.025, 0.05, 0.1 mg/kg, 60 min), and WAY 100,635 (0.4, 0.8, 1.6 mg/kg, 60 min).

Data Analysis

Stimulus control was determined as the number of sessions required for each subject to meet specified criteria of 80% correct responses in a minimum of 8 of 10 consecutive sessions. The number of sessions to criteria was determined from the first training session when both

levers were present. For each test compound, the mean (±SEM) percentage of drug-lever responses was determined for each dose. These data were analyzed with descriptive statistics and plotted in dose response curves for visual analysis. Tests in which an animal emitted less than 10 total responses were excluded from the analysis of percentage drug-lever selection. Full substitution by a test compound was defined as ≥ 80% drug-lever selection by any particular dose. Partial substitution was defined as drug-lever selection between 40 and 80%. Full antagonism by a test compound was defined as ≤ 20% drug-lever selection by any dose. Partial antagonism was defined as between 20 and 60% drug-lever selection.

Response rates were expressed as the number responses emitted per second during test sessions. For each test compound, the mean (±SEM) response rate was determined for each dose and these data were plotted in dose response curves. Response rates were included in statistical analyses regardless of the number of responses emitted. For each test compound, response rates were statistically analyzed using a one-way repeated-measures (RM) analysis of variance (ANOVA), followed by Dunnett's multiple comparisons to compare each test dose to the saline control. Graphical and statistical analyses were conducted using Prism GraphPad (Version 6.0).

CHAPTER III

RESULTS

Discrimination Acquisition

Stimulus control was established by 0.5 mg/kg MDPV with rats in *Experiment 1* within 26.7 (± 3.4, S.E.M.) training sessions (range 11-36). Rats used in the second experiment trained to discriminate 1.0 mg/kg MDPV from saline established stimulus control within 15.3 (± 1.2, SEM) training sessions (range 12-18).

Experiment 1

Stimulus Substitution

Dose-response curves generated from substitution tests with MDPV, MDMA, MDA and the optical isomers of MDMA or MDA are displayed in Figure 1. MDPV produced dose-dependent increases in MDPV-lever responses with minimal effects on response rate.

The MDMA and MDA enantiomers produced divergent results, with minimal MDPV-lever selection produced by (-)-MDMA or (-)-MDA, partial substitution by (+)-MDA (60%), and full substitution by (+)-MDMA (87%). The racemates produced full substitution with 83% MDPV-lever selection by (±)-MDMA and 89% by (±)-MDA. A dose-dependent decrease in response rate was observed with MDMA, MDA and their optical isomers. One-way RM ANOVA found a statistically significant reduction in response rate by (-)-MDMA [$F(3, 18) = 4.64$, $P < 0.05$], (+)-MDA [$F(3, 18) = 11.10$, $P < 0.001$], (-)-MDA [$F(3, 18) = 17.09$, $P < 0.0001$] as well as with (±)-MDMA [$F(3, 18) = 10.10$, $P < 0.001$] and (±)-MDA [$F(3, 18) = 13.14$, $P < 0.0001$]. Dunnett's multiple comparison tests indicated that the following doses and test compounds significantly reduced response rate compared to saline ($P < 0.05$): 1.5 mg/kg and 3.0

mg/kg (-)-MDMA; 0.75, 1.5 and 3.0 mg/kg (+)-MDA; 1.5 and 3.0 mg/kg (-)-MDA; 0.75, 1.5 and 3.0 mg/kg (±)-MDMA; 1.5 and 3.0 mg/kg (±)-MDA.

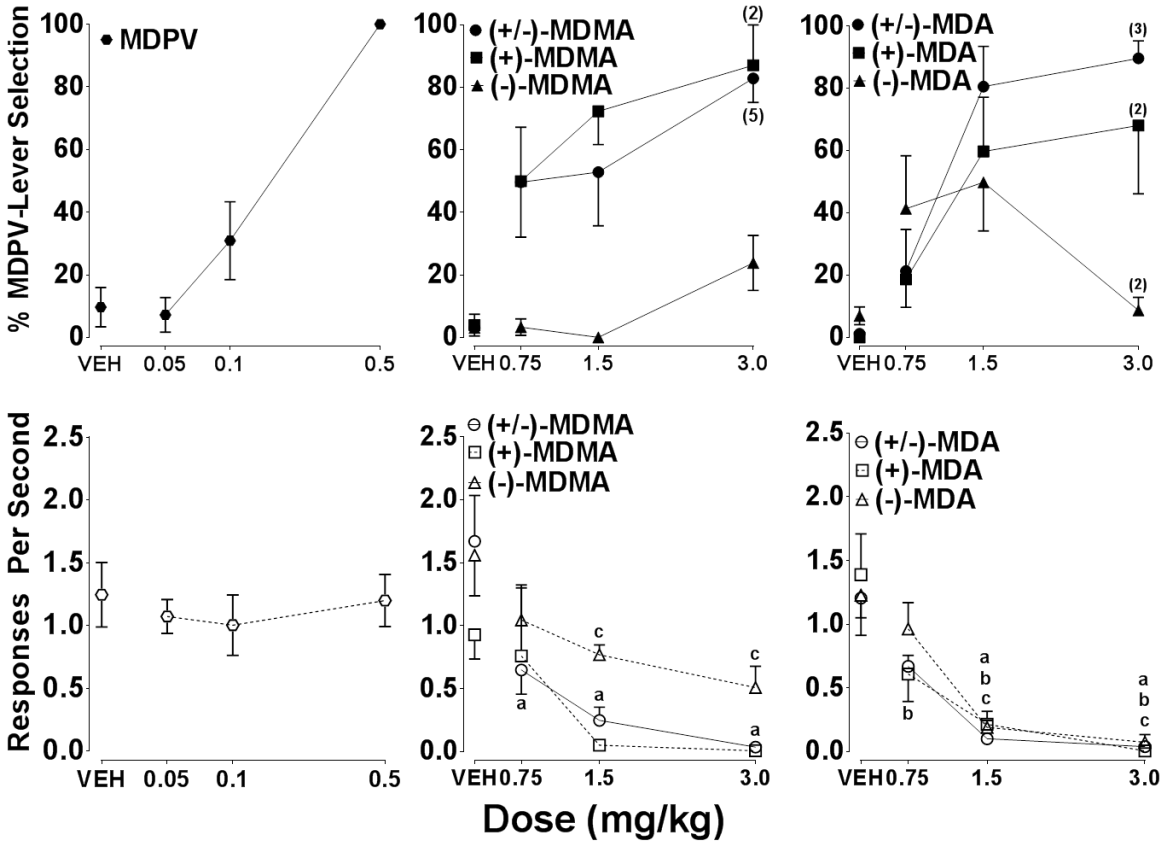

Figure 1. Dose-response curves determined from substitution tests with MDPV, MDMA, MDA and the optical isomers of MDMA and MDA in rats trained to discriminate 0.5 mg/kg MDPV from saline. Percentage MDPV-lever selection is depicted by closed symbols in the top row and response rate is shown as open symbols in the bottom row. Individual data points represent the group mean (± SEM). N=7 except where noted in parentheses at 3.0 mg/kg for some test compounds. Statistically significant Dunnett's tests compared to saline ($P < 0.05$) are indicated by a [(±)-MDMA or (±)-MDA], b [(+)-MDMA or (+)-MDA], or c [(-)-MDMA or (-)-MDA].

Cocaine fully substituted for MDPV at 5, 10 and 20 mg/kg and partially substituted at 2.5 mg/kg. The selective atypical DAT inhibitor, GBR 12909 and the NET/SERT inhibitor, desipramine, failed to substitute for MDPV at the doses tested. Dose-response curves generated

from stimulus substitution tests with these substances are displayed in Figure 2. A one-way RM ANOVA revealed a statistically significant effect of cocaine dose on response rate [$F(4, 16) = 3.87$, $P < 0.05$]. Dunnett's multiple comparison tests indicated 5 and 20 mg/kg cocaine lowered response rate compared to saline ($P < 0.05$). Neither GBR 12909 [$F(4, 20) = 1.04$, $P = 0.41$] nor desipramine [$F(3, 12) = 1.31$, $P = 0.32$] significantly reduced response rate.

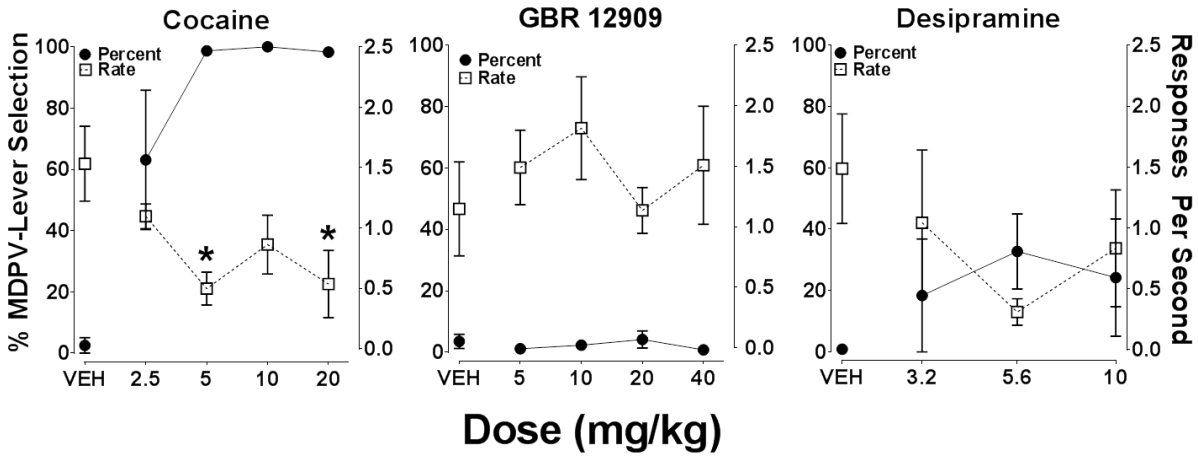

Figure 2. Dose-response curves generated from stimulus substitution tests with cocaine (n=5), GBR 12909 (n=6), and desipramine (n=5). Percentage MDPV-associated lever responses (closed symbols) refers to the left Y-axis and response rate (open symbols) refers to the right Y-axis. Individual points represent the group mean (± SEM). Statistically significant Dunnett's tests compared to saline are indicated by * ($P < 0.05$).

Stimulus Potentiation

Figure 3 displays the dose-response curves generated from stimulus potentiation tests with vehicle (n=5), 3.2 mg/kg desipramine (n=5), and 40 mg/kg GBR 12909 (n=4) pretreatment with each dose of MDPV. The vehicle + MDPV dose-response curve was nearly identical to the MDPV dose-response curve determined previously. Desipramine 3.2 mg/kg pretreatment with 0.1 mg/kg MDPV also produced complete substitution for MDPV (Rat-A: 18.52%, Rat-B: 100%, Rat-C: 100%, Rat-D: 100%, Rat-E: 95.45%). Pretreatment with 40 mg/kg GBR 12909

and MDPV 0.05 mg/kg produced complete stimulus substitution for MDPV, although only partial substitution was obtained with GBR 12909 + 0.1 mg/kg MDPV (n=3). Variability within subjects was observed with pretreatment of 40 mg/kg GBR 12909 and vehicle (Rat-B: 100%, Rat-C: 4.55%, Rat-D: 0%, Rat-E: 100%), as well as with GBR 12909 and 0.1 mg/kg of MDPV (Rat-A: 100%, Rat-B: 8.7%, Rat-C: 100%, Rat-D: n/a), which can be depicted from the error bars in Figure 3. Rat-D did not meet the response requirement during the test session with 40 mg/kg GBR 12909 + 0.1 mg/kg MDPV pretreatment that is needed to calculate the percentage of MDPV-appropriate lever selection. A RM ANOVA indicated no statistically significant changes in response rate compared to vehicle were produced by GBR 12909 + MDPV [$F(3, 9) = 0.73$, $P = 0.56$] or by desipramine + MDPV [$F(3, 12) = 1.95$, $P = 0.18$]. ED50 values were not determined due to an insufficient number of doses assessed to yield unambiguous results.

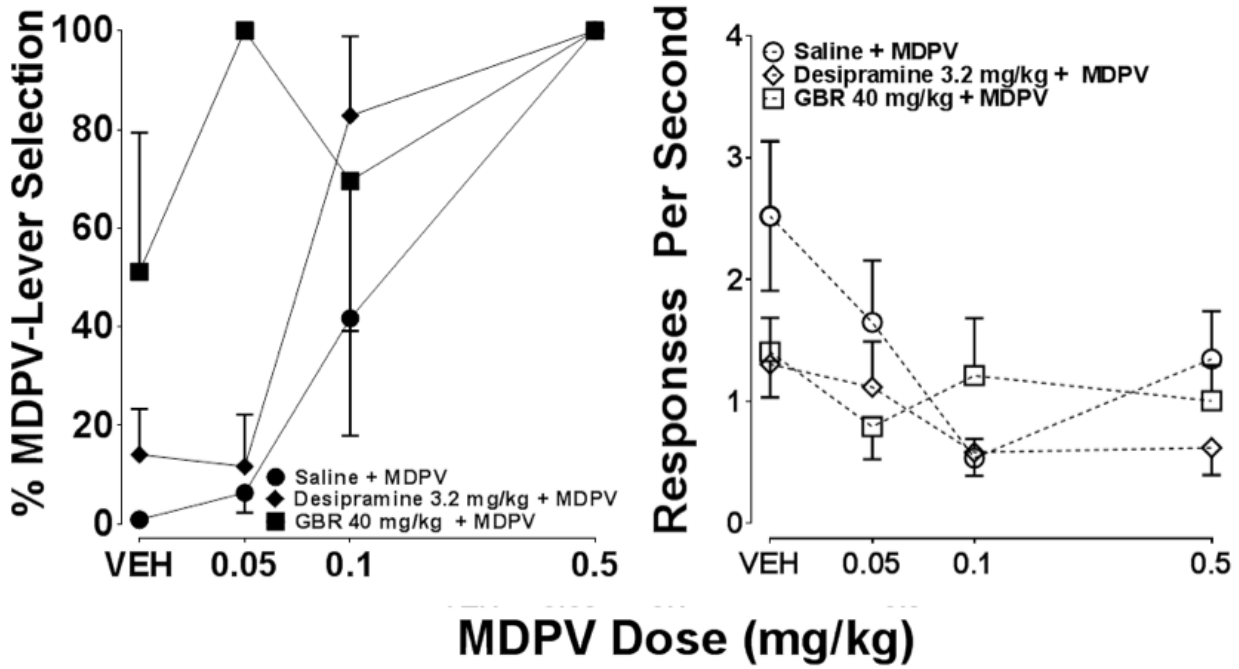

Figure 3. MDPV dose-response curves determined with saline (n=5), 40 mg/kg GBR 12909 (n=4, n=3 for percentage of MDPV-associated lever selection with 40 mg/kg GBR 12909 + MDPV at the 0.1 mg/kg dose), or 3.2 desipramine (n=5) pre-treatment. Percentage MDPV-lever

selection is depicted by closed symbols (left graph) and response rate is depicted as open symbols (rightward graph). Individual points represent group mean (± SEM).

Experiment 2

MDPV produced a dose-dependent increase in MDPV-lever responding, with full substitution produced by 0.3, and 1.0 mg/kg (training dose) (Figure 4). Dose-response curves generated with MDPV and each antagonist are displayed in Figure 5.

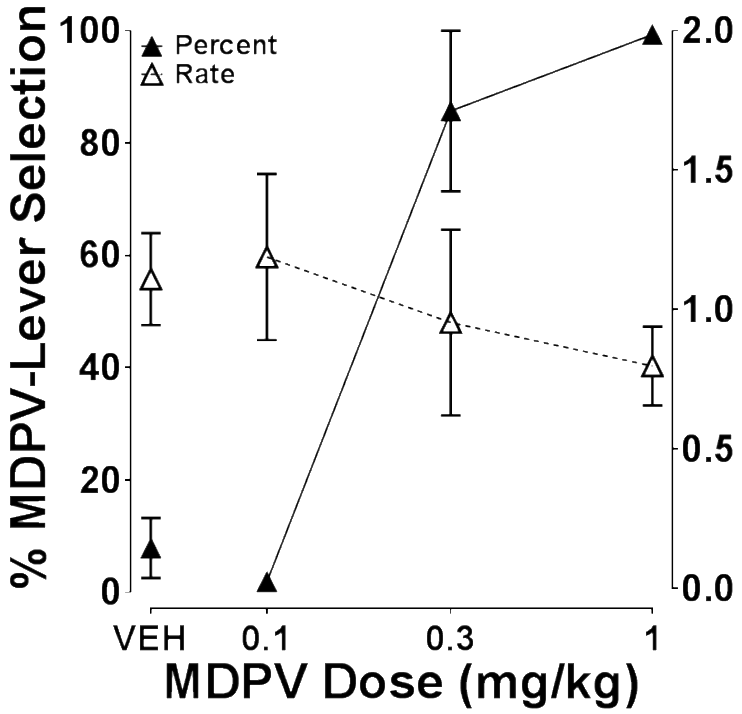

Figure 4. Dose-response curve for MDPV. Percentage MDPV-lever responses (closed symbols) refers to the left Y-axis and response rate (open symbols) refers to the right Y-axis. Individual points represent group mean (± SEM).

Dopamine Receptor Antagonists

The selective D_1 dopamine antagonist, Sch 23390 and the prototypical D_2 antagonist, haloperidol dose-dependently attenuated MDPV-lever selection. Full blockade of 1.0 mg/kg MDPV discrimination was observed with Sch 23390 0.1 mg/kg. A higher Sch 23390 dose (0.3

mg/kg) disrupted responding in nearly half of the 1.0 mg/kg MDPV-trained animals. Haloperidol produced only partial antagonism of 1.0 mg/kg MDPV at the highest dose tested (0.5 mg/kg).

A dose-dependent decrease in response rate was observed following tests with Sch 23390 and haloperidol. A one-way RM ANOVA found a statistically significant reduction in response rate following pretreatment with Sch 23390 and 1.0 mg/kg MDPV [$F(4, 24) = 4.17$, $P < 0.05$]. Dunnett's multiple comparison tests indicated that only the highest dose of Sch 23390 (0.3 mg/kg) reduced response rate to a level that was significantly different from response rate following saline pretreatment with 1.0 mg/kg MDPV ($P < 0.01$). One-way RM ANOVA tests did not find any statistically significant effects on response rates following the administration of haloperidol [$F(3, 18) = 0.82$, $P = 0.50$].

Serotonin Receptor Antagonists

The 5-HT_2 antagonist, pirenperone, the 5-HT_{2A} antagonist, MDL 100,907, and the 5-HT_{1A} antagonist WAY 100,635 did not alter 1.0 mg/kg MDPV discrimination. Response rate was not significantly different from control rates following pirenperone [$F(3, 12) = 2.23$, $P = 0.14$], MDL 100,907 [$F(3, 15) = 1.38$, $P = 0.29$], or WAY 100,635 [$F(3, 15) = 0.79$, $P = 0.52$].

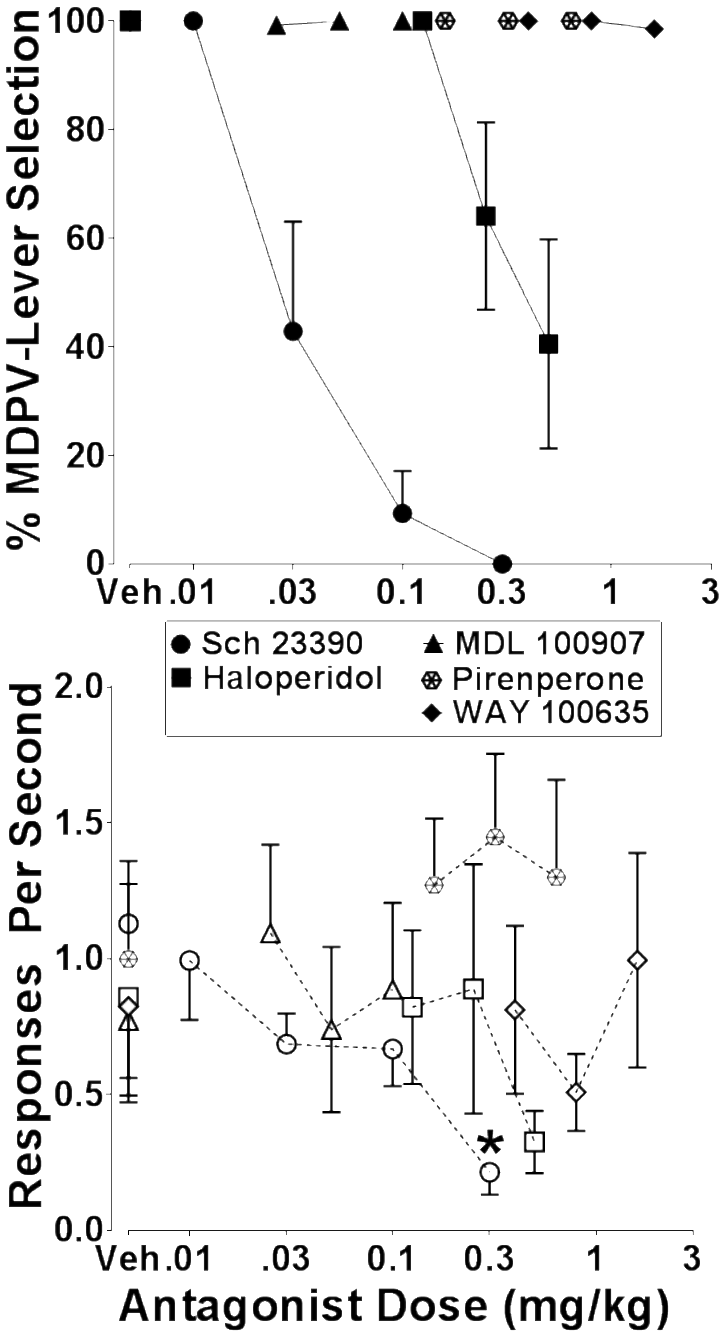

Figure 5. Stimulus antagonism tests with Sch 23390, haloperidol, pirenperone, MDL 100,907, and WAY 100,635 administered with 1.0 mg/kg MDPV are graphed above. Percentage of MDPV-appropriate lever responses (closed symbols) is represented in the top graph, with response rate (open symbols) represented in the lower graph. Individual points represent group mean (± SEM). Statistically significant Dunnett's tests compared to saline are indicated by * ($P < 0.05$).

CHAPTER IV

DISCUSSION

The current study examined the contribution of monoaminergic mechanisms in mediating the discriminative stimulus effects of the synthetic cathinone, MDPV through the assessment of stimulus substitution with 5-HT/DA releasers (MDMA, MDA and their optical isomers), a typical DAT inhibitor (cocaine), a highly selective atypical DAT inhibitor (GBR 12909), and a SERT/NET inhibitor (desipramine) in rats trained to discriminate 0.5 mg/kg MDPV (Experiment 1), and through the assessment of DA and 5-HT antagonists in rats trained to discriminate 1.0 mg/kg MDPV (Experiment 2).

Full substitution for MDPV was observed with cocaine, the racemates of MDMA and MDA, as well as (+)-MDMA. Only partial substitution was obtained with (+)-MDA at a dose that markedly reduced response rate, whereas (-)-MDMA and (-)-MDA produced virtually no substitution for MDPV. In consideration of previous evidence that the (+)-optical isomers of MDMA and MDA are more dopaminergic and the (-)-optical isomers are more serotonergic (Baker et al., 1995; Broadbent et al., 1992; Callahan & Appel, 1988; Glennon & Young, 1984), the current findings suggest MDPV discrimination is mediated to a greater extent by dopaminergic than serotonergic actions. Complete stimulus substitution by cocaine further supports this hypothesis and is consistent with previous reports regarding similarities in the interoceptive stimulus effects of MDPV and cocaine (Gatch et al., 2013; Gannon et al., 2016; Berquist & Baker, 2017). However, the absence of stimulus substitution by GBR 12909 indicates DAT inhibition alone is not sufficient to produce the MDPV cue.

The seemingly disparate results with cocaine and GBR 12909 in the current study may be due to cocaine's less selective, passive reuptake inhibition of DA, NE, and 5-HT compared to the

selective, atypical DAT inhibition by GBR 12909 (Andersen, 1989; Matecka et al., 1996). Curiously, GBR 12909 was reported to produce complete stimulus substitution for cocaine (Cunningham & Callahan 1991). However, rats can be trained to discriminate cocaine from GBR 12909 (Tella & Goldberg, 2001). Clearly, the training dose used to establish stimulus discrimination influences stimulus generalization to similar drugs.

Full substitution with (±)-MDMA for MDPV in the current study is consistent with previous findings reported by Fantegrossi et al. (2013) in a study of mice trained to discriminate 0.3 mg/kg MDPV, but contradict more recent findings reported by Berquist & Baker (2017) who trained rats to discriminate 0.3 mg/kg MDPV. Although the species and training procedures of the current study were similar to those reported by Berquist & Baker (2017), the training dose was slightly higher in the current study. A systematic evaluation of MDPV training dose may be required to determine if the training dose or other factors (e.g., order of test compounds assessed) account for the discrepant results.

As previously noted, the absence of MDPV stimulus substitution by GBR 12909 or by the tricyclic antidepressant, desipramine indicates that neither DAT nor NET/SERT inhibition alone is sufficient to produce the MDPV cue. Of particular interest, both GBR 12909 and desipramine pretreatment appeared to potentiate MDPV discrimination, indicative that both DAT and NET/SERT activities may modulate the actions of MDPV. Curiously, 40 mg/kg GBR 12909 pretreatment with vehicle tests produced an average of 51% MDPV-lever selection, substituting completely in two of the four animals tested. No dose of GBR 12909 produced even partial substitution when this substance was previously tested alone for substitution. However, the current results with GBR 12909 pretreatment should be considered with caution, as these tests were completed four months after the completion of initial GBR 12909 dose-response tests and

only four of the seven original animals were included in these tests (inconsistent responding, most likely due to age). It is possible that with more extensive training or with age, the animals developed greater sensitivity to the effects of GBR 12909. However, greater sensitivity to lower doses of MDPV were not evident, as the MDPV dose-response curve generated with vehicle pretreatments was nearly identical to the initial dose-response curve determined with MDPV at the beginning of the study. At the very least, these preliminary findings may serve to prompt further investigation. Results from the potentiation test conducted could be interpreted to suggest concurrent use of MDPV with monoamine reuptake inhibitors may have additive effects and perhaps an enhanced risk for abuse. Further investigation with a wider range of doses and with other selective monamine reuptake inhibitors are required to fully assess whether these substances have additive or synergistic effects with MDPV.

Results from Experiment 2 exhibiting dose-dependent attenuation and complete antagonism of MDPV discrimination by Sch 23390 indicate MDPV's stimulus effects are mediated primarily by dopamine's actions at D_1 receptors. Partial antagonism by haloperidol indicate D_2 DA receptors also contribute to these effects. In contrast, the absence of stimulus antagonism by pirenperone, MDL 100,907, and WAY 100,635 suggests a lack of involvement of 5-HT receptor mediation of the MDPV cue. These findings are consistent with previous reports that MDPV primarily acts as an uptake blocker at the dopamine transporter, with relatively weaker effects on serotonin release (Baumann et al., 2013; Simmler et al. 2013; Eshleman et al. 2013). It should be noted that the two highest doses of Sch 23390 (0.1 and 0.3 mg/kg) both attenuated 1.0 mg/kg MDPV discrimination and that only the highest dose (0.3 mg/kg) significantly reduced response rate. Additional tests with other selective dopamine receptor antagonists, preferably those that do not produce significant response rate disruption, are

warranted to determine if the response disruptive effects of Sch 23390 contributed to the attenuation of MDPV's discriminative stimulus effects.

In summary, considering the results of the two experiments described herein, complete stimulus substitution by the typical DAT inhibitor, cocaine and complete antagonism by DA receptor antagonists, but not 5-HT receptor antagonists, suggest MDPV's interoceptive stimulus effects are mediated predominantly by dopaminergic actions, with D_1 receptors contributing to these effects to a greater extent than D_2 receptors. However, we cannot rule out the possibility of serotonergic and/or noradrenergic modulation of these effects, given the substitution by MDMA and MDA and the potentiation by desipramine. Evaluation of additional DAT and NET inhibitors for potentiation of the MDPV cue and the assessment of additional antagonists, preferably with less disruptive effects on responding, may further assist in determining the precise neurochemical actions responsible for MDPV discrimination. Such knowledge can serve to inform medication discovery and development for the treatment of MDPV abuse.

REFERENCES

Aarde, S. M., Huang, P. K., Creehan, K. M., Dickerson, T. J., & Taffe, M. A. (2013). The novel recreational drug 3, 4-methylenedioxypyrovalerone (MDPV) is a potent psychomotor stimulant: self-administration and locomotor activity in rats. *Neuropharmacology*, *71*, 130-140.

Aarde, S. M., Huang, P. K., Dickerson, T. J., & Taffe, M. A. (2015). Binge-like acquisition of 3, 4-methylenedioxypyrovalerone (MDPV) self-administration and wheel activity in rats. *Psychopharmacology, 232*(11), 1867-1877.

Andersen, P. H. (1989). The dopamine uptake inhibitor GBR 12909: Selectivity and molecular mechanism of action. *European Journal of Pharmacology, 166*(3), 493-504.

Baker L.E. (2017) Hallucinogens in Drug Discrimination. In: Halberstadt A.L., Vollenweider F.X., Nichols D.E. (eds) *Behavioral Neurobiology of Psychedelic Drugs*. Current Topics in Behavioral Neurosciences, vol 36. Springer, Berlin, Heidelberg

Baker, L. E., Broadbent, J., Michael, E. K., Matthews, P. K., Metosh, C. A., Saunders, R. B., West, W. B., Appel, J. B. (1995). Assessment of the discriminative stimulus effects of the optical isomers of ecstasy (3, 4-methylenedioxymethamphetamine; MDMA). *Behavioural Pharmacology.*

Baumann, M. H., Partilla, J. S., Lehner, K. R., Thorndike, E. B., Hoffman, A. F., Holy, M., Rothman, R. B., Goldberg, S. R., Lupica, C. R., Sitte, H. H., Brandt, S. D., Tella, S. R., Cozzi, N. V., Schindler, C. W. (2013). Powerful cocaine-like actions of 3, 4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive 'bath salts' products. *Neuropsychopharmacology, 38*(4), 552.

Baumann, M. H., Bukhari, M. O., Lehner, K. R., Anizan, S., Rice, K. C., Concheiro, M., & Huestis, M. A. (2016). Neuropharmacology of 3, 4-methylenedioxypyrovalerone (MDPV), its metabolites, and related analogs. In *Neuropharmacology of New Psychoactive Substances (NPS)*, 93-117

Berquist, M. D., & Baker, L. E. (2017). Characterization of the discriminative stimulus effects of 3, 4-methylenedioxypyrovalerone in male Sprague-Dawley rats. *Behavioural Pharmacology, 28*(5), 394-400.

Broadbent, J., Appel, J. B., Michael, E. K., & Ricker, J. H. (1992). Discriminative stimulus effects of the optical isomers of 3, 4-methylenedioxyamphetamine (MDA). *Behavioural Pharmacology, 3*(5), 443-454.

Callahan, P. M., & Appel, J. B. (1988). Differences in the stimulus properties of 3, 4-methylenedioxyamphetamine and 3, 4-methylenedioxymethamphetamine in animals trained to discriminate hallucinogens from saline. *Journal of Pharmacology and Experimental Therapeutics, 246*(3), 866-870.

Cameron, K. N., Kolanos, R., Solis, E., Glennon, R. A., & De Felice, L. J. (2013). Bath salts components mephedrone and methylenedioxypyrovalerone (MDPV) act synergistically at the human dopamine transporter. *British Journal of Pharmacology, 168*(7), 1750-1757.

Centers for Disease Control and Prevention (CDC), 2011. Emergency department visits after use of a drug sold as "bath salts"—Michigan, November 13, 2010—March 31, 2011. *Morbidity and mortality weekly report 60, MMWR*, pp. 624–627. Retrieved from: https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6019a6.htm

Cunningham, K. A., & Callahan, P. M. (1991). Monoamine reuptake inhibitors enhance the discriminative state induced by cocaine in the rat. *Psychopharmacology*, *104*(2), 177-180.

Eshleman, A. J., Wolfrum, K. M., Hatfield, M. G., Johnson, R. A., Murphy, K. V., & Janowsky, A. (2013). Substituted methcathinones differ in transporter and receptor interactions. *Biochemical pharmacology*, *85*(12), 1803-1815.

Fantegrossi, W. E., Gannon, B. M., Zimmerman, S. M., & Rice, K. C. (2013). In vivo effects of abused 'bath salt' constituent 3, 4-methylenedioxypyrovalerone (MDPV) in mice: Drug discrimination, thermoregulation, and locomotor activity. *Neuropsychopharmacology*, *38*(4), 563.

Froberg, B. A., Levine, M., Beuhler, M. C., Judge, B. S., Moore, P. W., Engebretsen, K. M., Mckeown, N. J., Rosenbaum, C. D., Young, A. C., Rusyniak, D. E., & ACMT Toxicology Investigators Consortium (2015). Acute methylenedioxypyrovalerone toxicity. *Journal of Medical Toxicology*, *11*(2), 185-194.

Gannon, B. M., Galindo, K. I., Rice, K. C., & Collins, G. T. (2017). Individual differences in the relative reinforcing effects of 3, 4-methylenedioxypyrovalerone under fixed and progressive ratio schedules of reinforcement in rats. *Journal of Pharmacology and Experimental Therapeutics*, *361*(1), 181-189.

Gannon, B. M., Williamson, A., Suzuki, M., Rice, K. C., & Fantegrossi, W. E. (2016). Stereoselective effects of abused "bath salt" constituent 3, 4-methylenedioxypyrovalerone in mice: Drug discrimination, locomotor activity, and thermoregulation. *Journal of Pharmacology and Experimental Therapeutics*, *356*(3), 615-623.

Gatch, M. B., Taylor, C. M., & Forster, M. J. (2013). Locomotor stimulant and discriminative stimulus effects of "bath salt" cathinones. *Behavioural Pharmacology*, *24*, 437.

Glennon, R. A., & Young, R. (1984). MDA: An agent that produces stimulus effects similar to those of 3, 4-DMA, LSD and cocaine. *European Journal of Pharmacology*, *99*(2-3), 249-250.

Glennon, R. A., & Young, R. (Eds.). (2011). *Drug discrimination : applications to medicinal chemistry and drug studies*. Retrieved from https://ebookcentral-proquest-com.libproxy.library.wmich.edu

Goodwin, A. K., & Baker, L. E. (2000). A three-choice discrimination procedure dissociates the discriminative stimulus effects of d-amphetamine and (±)-MDMA in rats. *Experimental and clinical psychopharmacology*, *8*(3), 415.

Goodwin, A. K., Pynnonen, D. M., & Baker, L. E. (2003). Serotonergic–dopaminergic mediation of MDMA's discriminative stimulus effects in a three-choice discrimination. *Pharmacology Biochemistry and Behavior*, *74*(4), 987-995.

Harvey, E. L., & Baker, L. E. (2016). Differential effects of 3, 4-methylenedioxypyrovalerone (MDPV) and 4-methylmethcathinone (mephedrone) in rats trained to discriminate MDMA or a d-amphetamine MDMA mixture. *Psychopharmacology*, *233*(4), 673-680.

Harvey, E. L., Burroughs, R. L., & Baker, L. E. (2017). Effects of D1 and D2 receptor antagonists on the discriminative stimulus effects of methylenedioxypyrovalerone and mephedrone in male Sprague-Dawley rats trained to discriminate d-amphetamine. *Behavioural Pharmacology*, *28*(7), 586-589.

King, H.E., Wakeford, A., Taylor, W., Wetzell, B., Rice, K.C., & Riley, A.L. (2015a). Sex differences in 3,4-methylenedioxypyrovalerone (MDPV)-induced taste avoidance and place preferences. *Pharmacol Biochem Behav,* 137, 16-22.

King, H.E., Wetzell B., Rice, K.C., & Riley, A.L. (2015b). An assessment of MDPV-induced place preference in adult Sprague-Dawley rats. *Drug Alcohol Depend,* 146,116-119.

Matecka, D., Rothman, R. B., Radesca, L., de Costa, B. R., Dersch, C. M., Partilla, J. S., Pert, A., Glowa, J. R., Wojnicki, F. H., & Rice, K. C. (1996). Development of novel, potent, and selective dopamine reuptake inhibitors through alteration of the piperazine ring of 1-[2-(diphenylmethoxy) ethyl]-and 1-[2-[bis (4-fluorophenyl) methoxy] ethyl]-4-(3-phenylpropyl) piperazines (GBR 12935 and GBR 12909). *Journal of Medicinal Chemistry*, *39*(24), 4704-4716.

Rosenbaum, C. D., Carreiro, S. P., & Babu, K. M. (2012). Here today, gone tomorrow… and back again? A review of herbal marijuana alternatives (K2, Spice), synthetic cathinones (bath salts), kratom, Salvia divinorum, methoxetamine, and piperazines. *Journal of Medical Toxicology*, *8*(1), 15-32.

Ross, E. A., Reisfield, G. M., Watson, M. C., Chronister, C. W., & Goldberger, B. A. (2012). Psychoactive "bath salts" intoxication with methylenedioxypyrovalerone. *The American Journal of Medicine, 125*(9), 854-858.

Schindler, C. W., Thorndike, E. B., Goldberg, S. R., Lehner, K. R., Cozzi, N. V., Brandt, S. D., & Baumann, M. H. (2016). Reinforcing and neurochemical effects of the "bath salts" constituents 3, 4-methylenedioxypyrovalerone (MDPV) and 3, 4-methylenedioxy-N-methylcathinone (methylone) in male rats. *Psychopharmacology*, *233*(10), 1981-1990.

Simmler, L. D., Buser, T. A., Donzelli, M., Schramm, Y., Dieu, L. H., Huwyler, J., Chaboz, S., Hoener, M. C., & Liechti, M. E. (2013). Pharmacological characterization of designer cathinones in vitro. *British journal of pharmacology*, *168*(2), 458-470.

Spiller, H. A., Ryan, M. L., Weston, R. G., & Jansen, J. (2011). Clinical experience with and analytical confirmation of "bath salts" and "legal highs"(synthetic cathinones) in the United States. *Clinical Toxicology*, *49*(6), 499-505.

Stolerman, I.P., Mariathasan, E.A., White, J.A., & Olufsen, K.S. (1999). Drug mixtures and ethanol as compound internal stimuli. *Pharmacology Biochemistry and Behavior , 64 ,* 221–228.

Tella, S. R., & Goldberg, S. R. (2001). Subtle differences in the discriminative stimulus effects of cocaine and GBR-12909. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *25*(3), 639-656.

U.S. Department of Justice National Drug Intelligence Center. Situation Report Product Number 2011-S0787-004, Synthetic Cathinones (bath salts): An emerging domestic threat. 2011 Jul; Retrieved from http://www.justice.gov/archive/ndic/pubs44/44571/44571p.pdf.

Valente, M. J., De Pinho, P. G., de Lourdes Bastos, M., Carvalho, F., & Carvalho, M. (2014). Khat and synthetic cathinones: A review. *Archives of Toxicology*, *88*(1), 15-45.

Watterson, L. R., Kufahl, P. R., Nemirovsky, N. E., Sewalia, K., Grabenauer, M., Thomas, B. F., Marusich, J. A., Wegner, S., & Olive, M. F. (2014). Potent rewarding and reinforcing effects of the synthetic cathinone 3, 4-methylenedioxypyrovalerone (MDPV). *Addiction biology*, *19*(2), 165-174.

APPENDIX

WESTERN MICHIGAN UNIVERSITY

Institutional Animal Care and Use Committee

Date: February 24, 2016

To: Lisa Baker, Principal Investigator

From: Kathryn Eckler, DVM, Vice Chair

Re: IACUC Protocol Number 16-02-05

Your protocol entitled "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: February 23, 2017

1903 W. Michigan Ave., Kalamazoo, MI 49008-5456
PHONE: (269) 387-8293 FAX: (269) 387-8276

CAMPUS SITE: Walwood Hall