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Application of a Three-Lever Drug Discrimination Method to Differentiate the Interoceptive Stimulus Effects of 3, 4-Methylenedioxypyrovalerone and 4-Methylmethcathinone in Male Sprague-Dawley Rats

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APPLICATION OF A THREE-LEVER DRUG DISCRIMINATION METHOD TO DIFFERENTIATE THE INTEROCEPTIVE STIMULUS EFFECTS OF 3, 4-METHYLENEDIOXYPYROVALERONE AND 4-METHYLMETHCATHINONE IN MALE SPRAGUE-DAWLEY RATS

by

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Psychoactive “bath salts” represent a continuing drug abuse problem. The synthetic cathinones, 3, 4-methylenedioxypyrovalerone (MDPV) and 4-methylmethcathinone (4-MMC) are popular constituents of “bath salts” in the United States and the United Kingdom, respectively. Addiction to these substances has proven difficult to treat, possibly requiring targeted therapeutics. Drug discrimination is a preclinical assay that may aid in treatment development. Thus far, two-lever (drug vs no drug) discrimination studies have exhibited asymmetrical substitution patterns between 4-MMC and MDPV. Therefore, a three-lever discrimination method was employed in which 12 male Sprague-Dawley rats were trained to discriminate 0.5 mg/kg MDPV, 2.0 mg/kg 4-MMC, and saline vehicle. The discrimination was established within 39.8(± 3.9 S. E. M.) training sessions. Both MDPV and 4-MMC produced excellent stimulus control and dose-dependent increases in responding on the condition-appropriate lever. Response rate remained relatively stable across training and test sessions although was slightly higher under saline conditions. The present results indicate that 4-MMC and MDPV may produce substantially different subjective effects. Serotonergic mechanisms may contribute to these differential effects, but further experimentation is needed. The present data support a body of evidence that the three-lever drug discrimination design may be more sensitive to detecting differences between pharmacologically related substances.

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CHAPTER I

INTRODUCTION

Substance Abuse

Substance Use Disorder (SUD) is an ongoing public health concern that requires continuous research efforts to develop new treatments and therapies. According to the *Diagnostic and Statistical Manual of Mental Disorders,* 5th ed. (DSM-5), SUD is characterized by persistent behavioral, physical, social, and psychological impairments as they pertain to the use of drugs and/or alcohol (American Psychiatric Association, 2013). The DSM-5 categorizes SUD into six subtypes: alcohol, tobacco, cannabis, stimulant, hallucinogen, and opioid, and it encourages determining the severity of a SUD along a continuum, ranging from mild to severe (APA, 2013; Substance Abuse and Mental Health Services Administration, 2014).

A National Drug Threat Assessment published by the Drug Enforcement Agency (DEA) of the United States Department of Justice (USDOJ) states that deaths due to drug poisoning are at their highest rates ever (2016). At nearly 50,000 deaths in 2014 alone, drug poisoning outnumbers deaths due to suicide, homicide, motor vehicle accidents, and firearm related incidents (DEA, 2016). Reports from the Centers for Disease Control and Prevention (CDC) estimate drug overdose deaths at around 52,000 for 2015 and 64,000 for 2016 (Seth, Scholl, Rudd, & Bacon, 2018). Although opioid and heroin overdoses account for the largest portion of deaths (42,000 in 2016) and synthetic opioid overdoses represented the greatest increase in death rate (100% increase between 2015 and 2016), the rate of overdose deaths related to cocaine and other psychostimulants also increased 52.4% and 33.3%, respectively (Seth et al., 2018). Exacerbating the problem are reports that illicit drug use costs the American economy

approximately $193 billion between costs related to crime, healthcare, and lost productivity (USDOJ, 2011).

Synthetic Cathinones

Synthetic cathinones are psychomotor stimulants derived from cathinone, the active ingredient in the *Catha edulis* (khat) plant. Classified by the DEA (2016) as one of the two major classes of New Psychoactive Substances (NPS), synthetic cathinones provided the blueprint for how to circumvent laws, such as the Controlled Substances Act of 1970, and began a trend of "legal highs." Initially, synthetic cathinones were marketed as "bath salts," "research chemicals," "plant food," or simply "not for human consumption" in an effort to avoid detection by authorities (DEA, 2016; German, Fleckenstein, and Hanson, 2014). The DEA now states that this form of marketing for synthetic cathinones is subsiding in favor of names such as "Molly." This name is presumed to suggest that buyers are getting a pure form of another drug, 3, 4-methylenedioxymethamphetamine (MDMA). However, synthetic cathinones are common in drug formulations sold under the aforementioned street names (DEA, 2016). In general, "legal highs" may contain little of the active ingredient advertised on their labels, or the advertised active ingredient may be mixed with other substances (Baron, Elie, and Elie, 2011).

Several recent longitudinal studies monitoring drug use trends indicate that the use of synthetic cathinones is stable, if not declining (Johnston, Miech, O'Malley, Bachman, Schulenberg, and Patrick, 2018; European Monitoring Center for Drugs and Drug Addiction (EMCDDA), 2017). The DEA (2016) also notes that calls to poison control centers regarding synthetic cathinones toxicity peaked in 2011 and have declined every year since. However, several factors may be artificially deflating these numbers. Notably, the DEA (2016) suggests that emergency departments are now more familiar with the toxidrome associated with synthetic

cathinones. Thus, they may be able to treat overdoses better without the need to call poison control centers. More concerning, the new marketing strategies for synthetic cathinones may be confusing drug users into thinking they are consuming a different drug when they are in fact using a synthetic cathinone formulation (DEA, 2016).

As β-ketone analogues to the schedule II-controlled substance amphetamine, synthetic cathinones are similar structurally and pharmacologically to both amphetamine and related compounds like MDMA (Banks, Worst, and Sprague, 2014; for review, see Coppola and Mondola, 2012). For example, the popular synthetic cathinone constituent, 3, 4-methylenedioxypyrovalerone (MDPV), is a potent blocker of catecholamine transporters, such as the dopamine and norepinephrine transporters (DAT and NET, respectively), and it is significantly less potent as a blocker of the serotonin (5-HT) transporter (SERT) (Baumann et al., 2013). Conversely, another popular synthetic cathinone constituent, 4-methylmethcathinone (mephedrone, 4-MMC), functions as a substrate for monoamine transporters and is nearly equipotent at DAT, NET, and SERT (Baumann et al., 2012). Cameron, Kolanos, Solis, Glennon, and De Felice (2013a) and Cameron, Kolanos, Verkariya, De Felice, and Glennon (2013b) demonstrated the potency of MDPV as a dopamine reuptake inhibitor via human DAT inhibition whereas 4-MMC seemed to function as a dopamine releaser at hDAT. Although there are clear differences in mechanistic actions, the selectivity of MDPV to increase extracellular catecholamine concentrations is similar to that of amphetamine, cocaine, and methamphetamine (Baumann et al., 2013; Cameron et al., 2013a; Cameron et al., 2013b). Likewise, the relative non-selectivity of 4-MMC to increase extracellular concentrations of monoamines is similar to that of MDMA (Cameron et al., 2013a; Cameron et al., 2013b; Baumann et al., 2012). Considering these subtle differences in pharmacological effects, one may infer that these drugs

have similar, though not identical physiological and behavioral effects. These effects are examined in the following sections, with special attention paid to MDPV and 4-MMC.

3, 4-methylenedioxypyrovalerone (MDPV)

MDPV is one of the more popular synthetic cathinone constituents among drug users in the United States (Johnson and Johnson, 2014; Shanks, Dahn, Behonick, and Terrell, 2012). As previously mentioned, MDPV is a potent monoamine reuptake blocker with high selectivity for DAT and NET and low selectivity for SERT (Baumann et al., 2013). User reported subjective effects frequently associated with MDPV include feeling energetic/stimulated and a decreased appetite (Hall, Heyd, Butler, and Yarema, 2014; Johnson and Johnson, 2014). Less frequently reported, though still common, are feelings of increased sexual drive, euphoria, enhanced focus, and talkativeness (Johnson and Johnson, 2014). MDPV users also reported a number of adverse effects similar to those of other psychostimulants such as tachycardia, shortness of breath, paranoia, and chest pain (Hall et al., 2014; Johnson and Johnson, 2014). Myocardial infarction and death are also reported consequences of MDPV use following both binge patterns and standard recreational use (Wright, Cline-Parhamovich, Lajoie, Parsons, Dunn, and Ferslew, 2013).

The behavioral profile of MDPV is similar to that of other abused psychostimulants. Other than the user reported subjective effects previously mentioned, MDPV produces behaviors similar to other psychostimulants in animal models, especially preclinical trials of abuse liability. For example, MDPV produces locomotor sensitization following daily repeated administration (Berquist, Traxler, Mahler, and Baker, 2016) and it produces cross-sensitization to stimulants such as cocaine (Lopez-Arnau et al., 2017; Berquist et al., 2016). Conditioned place preference (CPP) is induced by MDPV, sometimes with greater preference shown for MDPV-paired

environments than environments paired with other addictive stimulants (King, Wetzell, Rice, and Riley, 2015; King, Wakeford, Taylor, Wetzell, Rice, and Riley, 2015; Karlsson, Andersson, Kronstrand, and Kugelberg, 2014). Adolescent exposure to MDPV also increases the rewarding properties of stimulants such as cocaine during adulthood (Lopez-Arnau et al., 2017).

A number of self-administration studies support that MDPV has potent addictive properties. For example, Simmons et al. (2016) demonstrated that MDPV is more readily self-administered than cocaine. Moreover, the same study found that rat ultra-sonic vocalizations, a measure of positive affect, were present in anticipation of the drug and persisted longer following drug administration when compared to cocaine. Watterson et al. (2014) demonstrated that MDPV treatment lowered the threshold for intracranial self-stimulation, a measure of drug reinforcing properties. They also showed that longer access to MDPV produced greater drug intake from animals. Aarde, Huang, Dickerson, and Taffe (2015) presented on the pattern of self-administration acquisition for MDPV finding that not only did rats engage in a binge-like self-administration pattern for this drug, but also that MDPV readily competed with natural reinforcement, despite that the study design allowed both MDPV self-administration and natural reinforcement. Gannon, Russell, Modi, Rice, and Fantegrossi (2017) found that mice would orally self-administer MDPV, although MDPV preference was not greater than that for water. Lastly, Gannon, Rice, and Collins (2017) demonstrated that both enantiomers of MDPV were readily self-administered at rates comparable to the racemate, although the R enantiomer was less potent than the S enantiomer. Coupled with DEA (2016) concerns regarding synthetic cathinones and reports of potentially serious side effects (Hall et al., 2014; Johnson and Johnson, 2014), these data clearly demonstrate the potential public health threat posed by MDPV.

4-methylmethcathinone (4-MMC, mephedrone)

Compared to MDPV, the synthetic cathinone constituent 4-MMC (also known as mephedrone) seems to be more popular among drug users in the United Kingdom (Winstock, Mitcheson, Ramsey, Davies, Puchnarewicz, and Marsden, 2011; Winstock, Mitcheson, Deluca, Davey, Corazza, and Shifano, 2010). Human reports of 4-MMC subjective effects, however, are similar to those subjective effects reported by MDPV users (Hall et al., 2014; Johnson and Johnson, 2014; Winstock et al., 2011). Such reports may be expected considering the similar pharmacological effects of MDPV and 4-MMC, noted previously. Like MDPV, 4-MMC use in humans may produce a number of toxicological effects typical of psychostimulants up to and including fatality (for review see Busardo, Kyriakou, Napoletano, Marinelli, and Zaami, 2015).

Similarities between 4-MMC and MDPV do not stop at pharmacology and subjective effects. Preclinical abuse liability studies reveal that many behavioral effects observed when MDPV is administered to animals also occur when 4-MMC is administered. For example, behavioral sensitization is evident after repeated daily treatment with 4-MMC (Berquist et al., 2016; Gregg, Tallarida, Reitz, McCurdy, and Rawls, 2013; Lisek et al., 2012) and prior treatment with 4-MMC enhances subsequent locomotor response to other psychostimulants such as cocaine (Berquist et al., 2016; Gregg, Tallarida, Reitz, and Rawls, 2013) and amphetamine (Berquist, Peet, and Baker, 2015). Conditioned place preference has been established following 4-MMC treatment (Karlsson et al., 2014; Lisek et al., 2012). Lastly, a number of experiments support that animals will readily self-administer 4-MMC (Nguyen, Grant, Creehan, Vandewater, and Taffe, 2016; Creehan, Vandewater, and Taffe, 2015; Motbey et al., 2013). Like MDPV, these studies provide a plethora of evidence that 4-MMC has abuse liability and could be considered a threat to public health.

Drug Discrimination and Study Rationale

Based on the evidence summarized above, clear similarities are noted between 4-MMC and MDPV with regard to behavioral and pharmacological effects and their abuse liability as predicted by preclinical assays. Nevertheless, these substances are structurally and pharmacologically distinct. When reviewed by Glennon (2014), the concluding remarks called for targeted therapeutic interventions for synthetic cathinones based on sometimes subtle, but still heterogeneous, effects of these drugs at neuroreceptors and neural pathways involved in addiction.

Drug discrimination is a preclinical assay with pharmacological specificity that may be helpful in identifying such therapeutic interventions. Discrimination, in the behavioral sense, is the act of differential responding to distinct stimuli. In drug discrimination, the distinct stimuli are presumed to be the interoceptive effects associated with centrally-mediated actions of a drug. Typically, drug discrimination is employed using two alternative responses that come under the control of a drug stimulus through a process of differential reinforcement. Subjects receive reinforcement for emitting a particular response (e.g., a left lever press) in the presence of a drug. On other occasions, they receive reinforcement for making an alternative response (e.g., a right lever press) in the absence of the drug. This is referred to as a drug - not drug (DN) discrimination. Although less common, subjects may be trained to discriminate two different drug stimuli, a design referred to as drug - drug (DD). Numerous researchers have investigated 4-MMC or MDPV using the former DN design (Varner et al. 2013; Gatch et al. 2013; Fantegrossi et al., 2013; Gannon et al., 2016; Harvey and Baker, 2016; Harvey et al., 2017; Berquist and Baker, 2017; Berquist et al., 2017). Gatch, Taylor, and Forster (2013), for example, demonstrated that both 4-MMC and MDPV substituted for the discriminative stimulus effects of

cocaine or methamphetamine at doses that did not disrupt responding. Similarly, Harvey, Burroughs, and Baker (2017) observed equivalent levels of substitution by MDPV and 4-MMC in rats trained to discriminate 0.5 mg/kg d-amphetamine. Conversely, 4-MMC substituted for MDMA, while MDPV only produced partial substitution in rats trained to discriminate 1.5 mg/kg MDMA (Harvey and Baker, 2016). Similarly, Berquist and Baker (2017) reported that MDMA produced minimal substitution in rats trained to discriminate 0.3 mg/kg MDPV.

Considered together, previous findings utilizing a DN discrimination suggest the interoceptive effects of MDPV and 4-MMC are similar but not identical. Moreover, 4-MMC appears to be more similar to MDMA. However, this may vary with species and training dose. Fantegrossi et al. (2013) found MDMA to fully substitute in mice trained to discriminate 0.3 mg/kg MDPV. Furthermore, in DN experiments directly comparing MDPV to 4-MMC, Berquist Thompson, and Baker (2017) found that MDPV fully substituted for a 1.0 mg/kg 4-MMC training dose, whereas only partial substitution was observed with MDPV for a 3.0 mg/kg 4-MMC training dose. In contrast MDMA fully substituted for both training doses of 4-MMC. In rats trained to discriminate 0.3 mg/kg MDPV, Berquist and Baker (2017) reported that 4-MMC produced only partial substitution at doses that severely disrupted responding.

Given the apparent asymmetrical substitution patterns between MDPV and 4-MMC, a more direct comparison of 4-MMC and MDPV in the drug discrimination design is warranted. Although a two-lever DD design could be used, some evidence suggests a three-lever discrimination between two drugs and a not drug stimulus (DDN) may be more sensitive to differentiating the effects of pharmacologically similar drugs. For example, Callahan and Appel (1990) discussed the difficulties of obtaining good stimulus control between lysergic acid diethylamide (LSD) and the pharmacologically similar substance, lisuride hydrogen maleate

when their laboratory used DD procedures. When a DDN procedure was attempted, however, the pair obtained much better stimulus control, as evidenced by steep generalization gradients with relatively little overlap in drug-lever responding. Additionally, Goodwin and Baker (2000) were able to dissociate the discriminative stimulus effects of d-amphetamine and MDMA using DDN procedures, although these drugs had previously exhibited asymmetrical substitution patterns, similar to those described regarding 4-MMC and MDPV. Therefore, the major goal of the present study was to determine if 4-MMC and MDPV cues could be established as distinct discriminative stimuli within the same animals. Subsequent goals were to assess other related substances for stimulus substitution to determine potential neuroreceptor targets that may contribute to differential stimulus cues between 4-MMC and MDPV.

CHAPTER II

EXPERIMENTAL METHODS

Animal Subjects

Twelve male Sprague-Dawley rats weighing 370-480g (Charles River Laboratories Inc., Kingston, NY, USA) completed the study. They were singly housed in polycarbonate cages with corncob bedding (ENVIGO, Madison, WI, USA) in a temperature and humidity controlled vivarium on a 12/12 light-dark cycle (lights on at 0500h). All animals had been previously used as saline controls in conditioned place preference experiments and were approximately four months old at the beginning of training. Animals were food restricted to maintain their weight at approximately 85-90% of their free-feeding weight, but they had access to water in their home cages *ad libitum.* Training procedures were initiated with six animals. An additional set of six animals was added after the first six met discrimination criteria. In total, 13 animals were used with two fatalities. The first occurred after only three sessions of discrimination training, so the animal was replaced. The second occurred after all but one training drug substitution test had been completed, so that animal was not replaced. All experimental procedures were in accordance with the *Guide for the Care and Use of Laboratory Animals* (2013) and were approved by the Institutional Animal Care and Use Committee of Western Michigan University.

Apparatus

All training and testing sessions were conducted in six computer-operated, standard three-lever rat operant chambers with retractable levers (ENV-100; MED Associates, St. Albans, VT, USA) contained within light- and sound- attenuating cabinets equipped with fans for ventilation. At the top of the rear wall, a houselight (28V) illuminated the chambers during all sessions. Three retractable levers were located on the front wall above a barred floor and below a

center food magazine. The two side levers were equidistant from the center food magazine while the center lever was located directly below the magazine. Food reinforcers consisted of 45 mg dustless precision purified pellets for rodents (F0021, Bioserv, Frenchtown, NJ, USA). All experimental events were recorded using Med-PC software version IV (Med-Associates, St. Albans, VT, USA) installed on a computer running Windows XP software.

Drugs

(±) 3, 4-methylenedioxypyrovalerone hydrochloride (HCl) (MDPV) and (±) 4-methylmethcathinone HCl (4-MMC) were provided by the National Institute on Drug Abuse Drug Supply Program (Bethesda, MD, USA). Each drug solution was prepared by dissolving the drug salt in 0.9% (wt/vol) bacteriostatic saline. All drug doses are expressed as the weight of salt. All drug injections were performed intraperitoneally (I.P., 1 ml/kg) with a 15-minute pre-session injection interval.

Operant Training Procedures

Magazine Training: Prior to any drug injections or lever press training, all animals were placed in one of the operant chambers for a 60-minute time period with no levers present. During that time, 45mg pellets were delivered on a fixed time 60-second (FT60sec) schedule to acclimate the animals to the location of food source and sound of pellet delivery. Animals were required to consume all of the pellets delivered during the FT60" in order to proceed to lever press training. This was accomplished in a single one-hour session.

Preliminary Training: Lever press training sessions began following magazine training and were conducted using errorless discrimination training procedures (i.e. only a single lever corresponding with the pre-session injection was present). During the first session, animals received no injection and only the center lever was present. Following the initial session, animals

received an I.P. injection of saline vehicle, 0.5 mg/kg MDPV, or 2.0 mg/kg 4-MMC 15 minutes prior to training and were replaced in their home cage. Following the presession interval, animals were placed in individual operant chambers for a 20-minute training session. Drug lever assignments were counterbalanced between the left and right levers among animals, but saline injections always corresponded with the center lever. The training doses of MDPV and 4-MMC were selected because those doses produced approximately equal levels of substitution for the discriminative stimulus maintained by d-amphetamine in a previous study (Harvey et al., 2017).

Food delivery was response-dependent under a fixed-ratio (FR) schedule of reinforcement. The animals began on a FR1 under each of the three training conditions. The FR requirement was gradually increased from FR1 to FR20 for each condition. Ratio increases in each condition were independent of one another. Each drug condition was presented a total of three times during lever press training, and the saline condition was presented four times. The order of delivery was SAL, 4-MMC, 4-MMC, SAL, MDPV, MDPV, SAL, 4-MMC, MDPV, SAL. All animals reached FR20 under each condition without the need to extend this training order.

Discrimination Training: During discrimination training, animals received an I.P. injection of 4-MMC, MDPV, or saline 15 minutes prior to the beginning of the session and then replaced in their home cages. Following the 15-minute pre-session interval, animals were placed in individual operant chambers. All three levers were present during discrimination training. Considering the enhanced difficulty of the three-lever discrimination task compared to preliminary training, the FR requirement during discrimination training was lowered from a FR20 to a FR10 schedule of reinforcement to facilitate higher levels of responding. Injection order was pseudorandom under the stipulation that the same condition could not be presented

more than two sessions in a row and drug conditions could not be presented more than two sessions in a row (i.e., following two drug sessions, the next session had to be a vehicle session). Lever assignments remained constant and were counterbalanced within groups. Criteria for discrimination called for each animal to reach ≥80% condition-appropriate responding for the first FR and the remainder of the session for at least eight of ten consecutive discrimination training sessions.

Substitution Tests: Substitution tests commenced once each animal reached the aforementioned discrimination training criteria. Between testing sessions, an animal had to complete no less than one discrimination training session on each of the three conditions wherein the animal met the criteria of ≥80% condition-appropriate responding on both the first FR and for the entire session. If an animal's performance fell below these criteria, discrimination training continued until these criteria were met. Doses of test compounds were counterbalanced across subjects. Substitution tests typically occurred one to two times per week. Test sessions were conducted with the substances listed in Table 1:

Table 1

Compounds for Substitution Tests

Test Compound	Drug Dose (mg/kg)
4-MMC HCl	0.0, 0.25, 0.5, 1.0, 2.0, 3.0
MDPV HCl	0.0, 0.063, 0.125, 0.25, 0.5, 1.0

*Substitution tests were conducted first with 4-MMC and then with MDPV. The training dose of 4-MMC was 2.0 mg/kg and the training dose of MDPV was 0.5 mg/kg. To the best of the experimenter's ability, the order of test doses was counterbalanced

Test sessions were identical to discrimination training sessions in that animals were injected I. P. with the test solution 15 minutes prior to the beginning of the session and then replaced in their home cages. After the pre-session interval had elapsed, animals were placed into the operant chamber and the session began. Test sessions concluded after an animal completed a

FR10 on any lever or after 20 minutes had elapsed. Test sessions were conducted under extinction conditions (i.e. no reinforcement followed FR completion).

Data Analysis

Response accuracy was determined by calculating the percentage of total responses emitted on each lever prior to the delivery of the first reinforcer of each training session. Response rate was calculated by dividing the total number of responses emitted during a training session by 1200 seconds. Group mean (±S.E.M.) response accuracy and group mean (±S.E.M.) response rate were plotted for each training condition over the first 55 discrimination training sessions. Acquisition of drug stimulus control was determined by the number of discrimination training sessions required to meet the aforementioned performance criteria. Stimulus control acquisition was analyzed as the group mean (±S.E.M.) number of sessions required to meet discrimination criteria.

For the analysis of substitution test results, the percentage of MDPV, 4-MMC or saline-lever responses was calculated by dividing the number of responses on each lever by the total number of responses during the test session. These data were analyzed by calculating the group mean (±S.E.M.) percent of responses allocated to each lever following each test dose. Response rate was recorded during each session as the number of responses emitted per second. Response rate in drug substitution tests was analyzed by a one-way repeated measures analysis of variance (ANOVA) followed by Tukey post-hoc comparisons.

CHAPTER III

RESULTS

Discrimination Acquisition

The main objective of the present study was to determine if rats could learn to discriminate the interoceptive stimulus effects of MDPV from those of 4-MMC using a three-lever discrimination procedure. Response accuracy during the discrimination acquisition phase is displayed in Figure 1, with each stimulus condition plotted separately. These data are depicted in separate graphs for each training cohort because order of training stimulus conditions was slightly different for the two training cohorts. However, both cohorts were combined when determining the mean number of sessions to criterion. Briefly, stimulus control was established under all three training conditions, with percentage of responses on the appropriate lever gradually increasing as training progressed. The mean number of sessions to establish stimulus control for all three conditions was 39.8 ±3.9 (S.E.M.) (Range: 22-67).

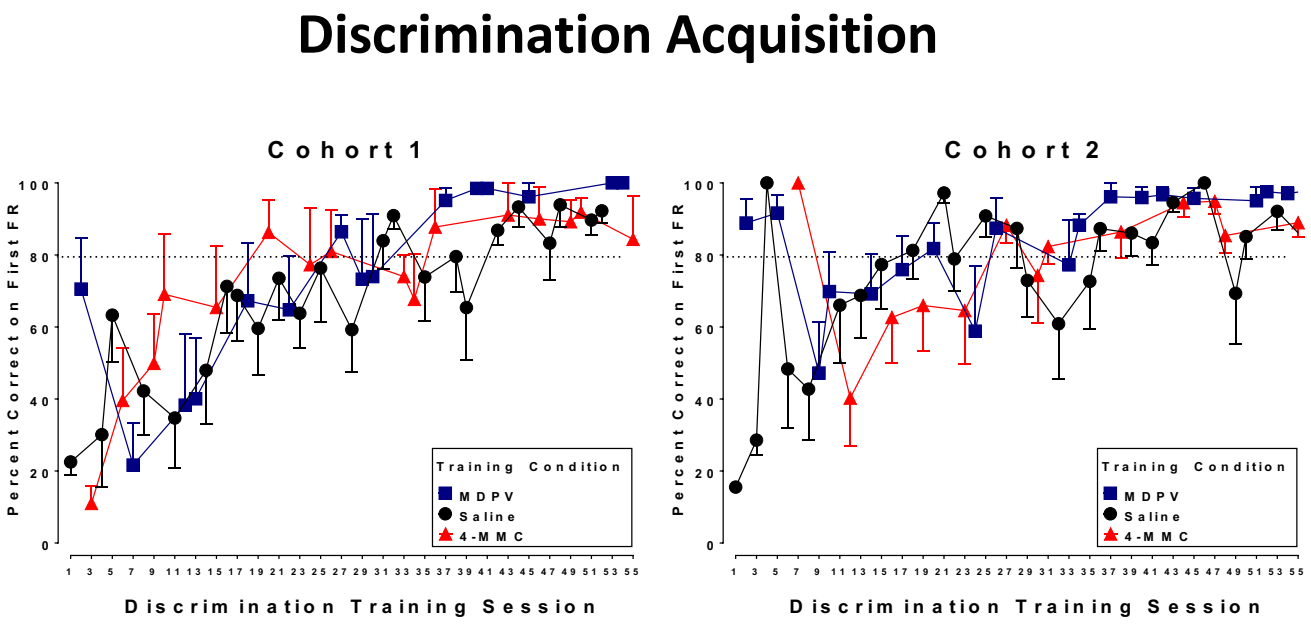

Figure 1. Response accuracy during the discrimination acquisition phase in 12 rats (n=6 per cohort) trained to discriminate 0.5 mg/kg MDPV, 2.0 mg/kg 4-MMC, and saline vehicle. Data points depict the group mean (± S. E. M.) percentage of responses on each lever up until the completion of the first FR10 during the first 55 discrimination training sessions (n=6 per cohort).

Overall response rate during the acquisition phase is plotted in Figure 2. Response rate was relatively stable across all training conditions during the acquisition period and was slightly higher when saline was administered than when 4-MMC or MDPV were administered. Four animals (B2, B5, B7, and B8) were returned briefly to errorless training conditions during discrimination training session number 18, 16, 43, and 37, respectively, because they did not earn any reinforcers during a number of sessions.

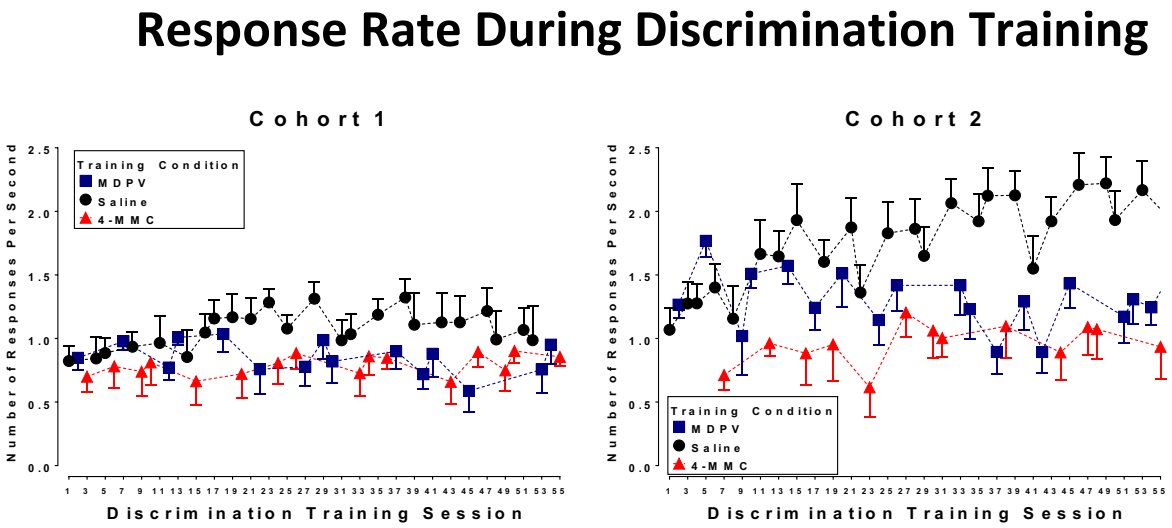

Figure 2. Response rate during the acquisition phase of discrimination training reported as the group mean responses per second (± S. E. M.) for each training condition (n=6 per cohort).

Substitution Tests

Dose response curves determined from 4-MMC and MDPV substitution tests are shown in Figure 3. During these tests, responses were allocated to the condition-appropriate lever for

both 4-MMC and MDPV in a dose-dependent manner, whereas dose-dependent decreases in saline-lever responses were observed. Very few responses were emitted on the MDPV lever during 4-MMC substitution tests and vice versa.

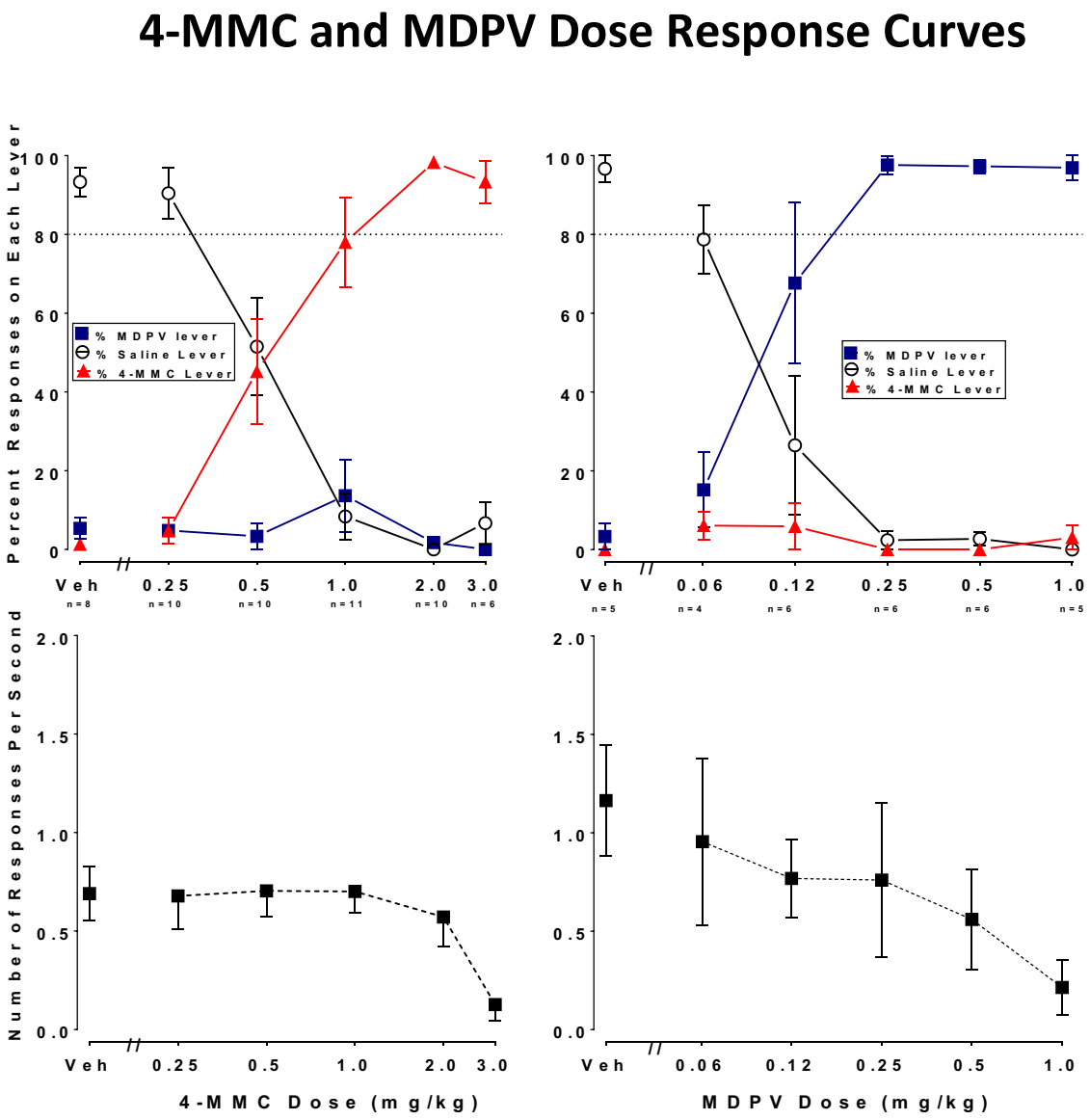

Figure 3. Dose response curves determined from substitution tests with 4-MMC (left) and MDPV (right). Upper graphs depict the group mean (± S. E. M.) percentage of responses on each lever. Lower graphs depict the group mean (± S. E. M.) response rate expressed as the total

number of responses on any lever per second. The number of animals included in each group mean is shown below the dose labels on the X axis in the upper graphs.

CHAPTER IV

DISCUSSION

The specific aim of the present experiment was to determine if rats could learn to discriminate both 4-MMC and MDPV from saline injections in a three-lever drug discrimination procedure. To that end, this experiment was successful in that stimulus control was readily established by these substances. Of particular interest, stimulus control was established in approximately the same number of sessions required for rats to learn a two-lever discrimination between either of these drugs and saline (Berquist and Baker, 2017; Berquist et al., 2017). The speed with which stimulus control was established in the present experiment is notable because previous three-lever discrimination studies with pharmacologically-similar drugs required nearly twice as many sessions, on average, for stimulus control to be established (Goodwin and Baker, 2000; Callahan and Appel, 1990). Some evidence from studies with human subjects suggests that errorless discrimination procedures produce faster learning than traditional trial and error procedures (Schilmoeller, Schilmoeller, Etzel, and LeBlanc, 1979). Berquist et al. (2017) in part attributed the speed with which animals learned a two-lever discrimination (4-MMC versus saline) to errorless training procedures when comparing their results to those of Varner et al. (2013) who used traditional trial and error training procedures. However, the errorless training methods employed in the present three-lever discrimination were comparable to those of Goodwin and Baker (2000) and Callahan and Appel (1990). As such, differences among these studies in the number of sessions to obtain stimulus control are likely not due to differential training methods.

Dose response curves with each training drug provide further support that 4-MMC and MDPV produce differential interoceptive stimulus effects, as substitution tests with each drug

produced virtually no responses on the other drug lever. Inasmuch as drug discrimination is a predictive model of subjective drug effects, the current findings indicate 4-MMC and MDPV produce substantially different subjective effects. Systematic controlled studies comparing the subjective effects of these substances in humans are currently nonexistent. However, a random examination of 4-MMC and MDPV user reports obtained from the website Erowid.org support the current findings that their subjective effects are distinct. For example, details from the accounts, Harmoniousaccord (2009), Jovialla (2010), and Smushy (2010), on the Erowid website regarding purported 4-MMC use indicate the presence of mild to moderate hallucinogenic effects from this drug. Two of these accounts, Harmoniousaccord and Smushy, directly compared 4-MMC to MDMA. In contrast, reports from Erowid authors, GewaltHaber (2011) and Brain_Damage (2017) regarding purported MDPV use described the effects as distinctly different from those of MDMA. These reports also did not indicate hallucinations as an effect of MDPV, whereas visual hallucinations seemed to be a relatively common effect reported by 4-MMC user reports. It should be noted at this point, however, that Erowid user experiences are not controlled and are categorized based on the drugs a user suspects they consumed.

The subjective effects of 4-MMC may also vary with dose. As noted in a recent report by Berquist et al. (2017), substitution for 4-MMC with MDPV and other related compounds is dependent on the training dose. In that study, rats trained to discriminate 1.0 mg/kg 4-MMC generalized to MDPV and several other psychostimulants, whereas rats trained to discriminate 3.0 mg/kg 4-MMC generalized to only 4-MMC and MDMA. Therefore, the extent to which the stimulus effects of 4-MMC and MDPV differ may depend on the particular training dose selected. The doses selected for the current study produced equivalent levels of drug-appropriate

responding in rats trained to discriminate d-amphetamine (Harvey et al., 2017). The fact that these doses were discriminated readily is noteworthy.

These preliminary data indicate a role for 5-HT in the discriminative stimulus cue mediated by 4-MMC, as serotonergic effects seem to be the most outstanding pharmacological difference between MDPV and 4-MMC. This supports previous research evidence that 4-MMC substituted for MDMA, a more potent serotonergic drug than other psychostimulants (Harvey and Baker, 2016). Further investigation involving substitution tests with other serotonergic substances is required to fully evaluate this proposition. Furthermore, dopaminergic mechanisms may also be involved in the 4-MMC discriminative stimulus cue as evidenced by stimulus generalization blockade when the D_1 antagonist, Schering 39116, was given as a pretreatment prior to 4-MMC substitution tests for the dopaminergic psychostimulant, d-amphetamine (Harvey et al., 2017).

Ongoing investigation will further clarify the role of various neuroreceptors and their subtypes in the discriminative stimulus effects of both 4-MMC and MDPV. At present, it may be hypothesized that substances with predominantly dopaminergic activity, such as amphetamine and methamphetamine, may substitute for MDPV while serotonergic substances, such as LSD and MDMA, may substitute for 4-MMC. Such results would be consistent with prior research (Berquist and Baker, 2017; Berquist et al., 2017; De Large, Erwin, and Winsaur, 2017; Harvey et al., 2017; Harvey and Baker, 2016), but remain to be demonstrated by ongoing research. Receptor specific antagonist tests would aid in determining the relative contributions of various neuroreceptors to the discriminative stimulus effects of both 4-MMC and MDPV. These types of tests may also be more well suited to answer the question of whether the differential

discriminative stimulus effects of these two drugs rely on dopaminergic versus serotonergic differences.

Also warranted are studies to investigate whether the optical isomers of 4-MMC and MDPV have differential discriminative stimulus effects. A growing body of evidence suggests that the reinforcing and rewarding properties of MDPV and 4-MMC may vary among their enantiomers (Gannon et al., 2017; Philogene-Khalid et al., 2017). Determination of discriminative stimulus effects may be crucial for the development of targeted therapeutics to treat addiction to either 4-MMC or MDPV. Some evidence suggests that the S enantiomer of 4-MMC reduces anxiety and depressant-like effects observed in cocaine and MDPV abstinent rats (Philogene-Khalid, Hicks, Reitz, Liu-Chen, and Rawls, 2017). Thus, if 4-MMC isomers have differential reinforcing properties, full or partial substitution for a racemic training dose may indicate a potential target for therapeutic intervention. MDPV enantiomers would not likely be ideal therapeutic targets as both the S and R enantiomers support high levels of drug self-administration, although there are differences in potency (Gannon et al., 2017). Such a research project would require characterization of toxicological effects associated with each isomer.

In conclusion, male Sprague-Dawley rats readily learned to discriminate 4-MMC and MDPV from saline in a three-lever drug discrimination procedure. Although further experimental confirmation is required, preliminary data indicate that serotonergic differences may account, at least in part, for differential discriminative stimulus effects between these drugs. Due to the relative speed with which animals acquired the discrimination in the present experiment, the current research further supports a body of evidence that the three-lever drug discrimination design is more sensitive than comparable two-lever discrimination designs in detecting differences between pharmacologically similar substances.

REFERENCES

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.

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.). Arlington, VA: American Psychiatric Publishing.

Banks, M. L., Worst, T. J., & Sprague, J. E. (2014). Synthetic cathinones and amphetamine analogues: What's the rave about? *Journal of Emergency Medicine,46*(5), 632-642.

Baron, M., Elie, M., & Elie, L. (2011). An analysis of legal highs-do they contain what it says on the tin? *Drug Testing and Analysis,3*(9), 576-581.

Baumann, M. H., Ayestas, M. A., Jr., Partilla, J. S., Sink, J. R., Shulgin, A. T., Daley, P. F., . . . Cozzi, N. V. (2012). The designer methcathinone analogs, mephedrone and methylone, are substrates for monoamine transporters in brain tissue. *Neuropsychopharmacology, 37*(5), 1192-1203.

Baumann, M. H., Partilla, J. S., Lehner, K. R., Thorndike, E. B., Hoffman, A. F., Holy, M., . . . Schindler, C. W. (2013). Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive 'bath salts' products. *Neuropsychopharmacology,38*(4), 552-562.

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.

Berquist, M. D., Peet, M. M., & Baker, L. E. (2015). Behavioral sensitization following concurrent exposure to mephedrone and D-amphetamine in female mice. *Behavioural Pharmacology,26*, 180-183.

Berquist, M. D., Thompson, N. A., & Baker, L. E. (2017). Evaluation of training dose in male Sprague-Dawley rats trained to discriminate 4-methylmethcathinone. *Psychopharmacology,234*(21), 3271-3278.

Berquist, M. D., Traxler, H. K., Mahler, A. M., & Baker, L. E. (2016). Sensitization to the locomotor stimulant effects of "bath salt" constituents, 4-methylmethcathinone (4-MMC) and 3,4-methylenedioxypyrovalerone (MDPV), in male Sprague-Dawley rats. *Drug and Alcohol Dependence,164*, 128-134.

Brain_Damage. "Not an MDMA Substitute: An Experience with MDPV (exp110196)". Erowid.org. Mar 30, 2017. erowid.org/exp/110196

Busardo, F. P., Kyriakou, C., Napoletano, S., Marinelli, E., & Zaami, S. (2015). Mephedrone related fatalities: A review. *European Review for Medical and Pharmacological Sciences,19*, 3777-3790.

Callahan, P. M., & Appel, J. B. (1990). Differentiation between the stimulus effects of (+)-lysergic acid diethylamide and lisuride using a three-choice, drug discrimination procedure. *Psychopharmacology,100*(1), 13-18.

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

Cameron, K., Kolanos, R., Verkariya, R., De Felice, L., & Glennon, R. A. (2013b). Mephedrone and methylenedioxypyrovalerone (MDPV), major constituents of "bath salts," produce opposite effects at the human dopamine transporter.*Psychopharmacology, 227*(3), 493-499.

Coppola, M., & Mondola, R. (2012). Synthetic cathinones: Chemistry, pharmacology and toxicology of a new class of designer drugs of abuse marketed as “bath salts” or “plant food”. *Toxicology Letters,211*, 144-149.

Creehan, K. M., Vandewater, S. A., & Taffe, M. A. (2015). Intravenous self-administration of mephedrone, methylone and MDMA in female rats. *Neuropharmacology,92*, 90-97.

Delarge, A. F., Erwin, L. L., & Winsauer, P. J. (2017). Atypical binding at dopamine and serotonin transporters contribute to the discriminative stimulus effects of mephedrone. *Neuropharmacology,119*, 62-75.

Drug Enforcement Agency. (2016). National Drug Threat Assessment 2016. *United States Department of Justice*.

European Monitoring Centre for Drugs and Drug Addiction (2017), *European Drug Report 2017: Trends and Developments*, Publications Office of the European Union, Luxembourg.

Gannon, B. M., Rice, K. C., & Collins, G. T. (2017). Reinforcing effects of abused ‘bath salts’ constituents 3,4-methylenedioxypyrovalerone and α-pyrrolidinopentiophenone and their enantiomers. *Behavioural Pharmacology,28*(7), 578-581.

Gannon, B. M., Russell, L. N., Modi, M. S., Rice, K. C., & Fantegrossi, W. E. (2017). Effects of orally self-administered bath salt constituent 3,4-methylenedioxypyrovalerone (MDPV) in mice. *Drug and Alcohol Dependence,179*, 408-415.

Gatch, M. B., Taylor, C. M., & Forster, M. J. (2013). Locomotor stimulant and discriminative stimulus effects of ‘bath salt’ cathinones. *Behavioural Pharmacology, 24*(5-6), 437-447.

German, C. L., Fleckenstein, A. E., & Hanson, G. R. (2014). Bath salts and synthetic cathinones: An emerging designer drug phenomenon. *Life Sciences,97*(1), 2-8.

GewaltHaber. "The Full Ride: An Experience with MDPV (exp90171)". Erowid.org. May 12, 2011. erowid.org/exp/90171

Glennon, R. A. (2014). Bath salts, mephedrone, and methylenedioxypyrovalerone as emerging illicit drugs that will need targeted therapeutic intervention. *Emerging Targets & Therapeutics in the Treatment of Psychostimulant Abuse Advances in Pharmacology,*581-620.

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), 415-423.

Gregg, R. A., Tallarida, C. S., Reitz, A., Mccurdy, C., & Rawls, S. M. (2013). Mephedrone (4-methylmethcathinone), a principal constituent of psychoactive bath salts, produces behavioral sensitization in rats. *Drug and Alcohol Dependence,133*(2), 746-750.

Gregg, R. A., Tallarida, C. S., Reitz, A. B., & Rawls, S. M. (2013). Mephedrone interactions with cocaine: Prior exposure to 'bath salt' constituent enhances cocaine-induced locomotor activation in rats. *Behavioural Pharmacology,24*(8), 684-688.

Hall, C., Heyd, C., Butler, C., & Yarema, M. (2014). ''Bath salts'' intoxication: A new recreational drug that presents with a familiar toxidrome. *Canadian Journal of Emergency Medicine,16*(2), 171-176.

Harmoniousaccord. "Rubbery Abandon: An Experience with 4-Methylmethcathinone (exp78080)". Erowid.org. May 27, 2009. erowid.org/exp/78080

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 methylendioxypyrovalerone and mephedrone in male Sprague-Dawley rats trained to discriminate D-amphetamine. *Behavioural Pharmacology,28*(7), 586-589.

Johnson, P. S., & Johnson, M. W. (2014). Investigation of "bath salts" use patterns within an online sample of users in the United States. *Journal of Psychoactive Drugs,46*(5), 369-378.

Johnston, L. D., Miech, R. A., O'Malley, P. M., Bachman, J. G., Schulenberg, J. E., & Patrick, M. E. (2018). Monitoring the Future national survey results on drug use: 1975-2017:

Overview, key findings on adolescent drug use. Ann Arbor: Institute for Social Research, The University of Michigan.

Jovialla. "Sparkle: An Experience with 4-Methylmethcathinone (Mephedrone) (exp83324)". Erowid.org. Mar 28, 2010. erowid.org/exp/83324

Karlsson, L., Andersson, M., Kronstrand, R., & Kugelberg, F. C. (2014). Mephedrone, Methylone and 3,4-Methylenedioxypyrovalerone (MDPV) Induce Conditioned Place Preference in Mice. *Basic & Clinical Pharmacology & Toxicology,115*(5), 411-416.

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

King, H. E., Wetzell, B., Rice, K., & Riley, A. (2015). An assessment of MDPV-induced place preference in adult Sprague-Dawley rats. *Drug and Alcohol Dependence,156*.

Lisek, R., Xu, W., Yuvasheva, E., Chiu, Y., Reitz, A. B., Liu-Chen, L., & Rawls, S. M. (2012). Mephedrone ('bath salt') elicits conditioned place preference and dopamine-sensitive motor activation. *Drug and Alcohol Dependence, 126*(1-2), 257-262.

López-Arnau, R., Luján, M. A., Duart-Castells, L., Pubill, D., Camarasa, J., Valverde, O., & Escubedo, E. (2017). Exposure of adolescent mice to 3,4-methylenedioxypyrovalerone increases the psychostimulant, rewarding and reinforcing effects of cocaine in adulthood. *British Journal of Pharmacology,174*(10), 1161-1173.

Motbey, C. P., Clemens, K. J., Apetz, N., Winstock, A. R., Ramsey, J., Li, K. M., . . . Mcgregor, I. S. (2013). High levels of intravenous mephedrone (4-methylmethcathinone) self-administration in rats: Neural consequences and comparison with methamphetamine. *Journal of Psychopharmacology,27*(9), 823-836.

Nguyen, J. D., Grant, Y., Creehan, K. M., Vandewater, S. A., & Taffe, M. A. (2016). Escalation of intravenous self-administration of methylone and mephedrone under extended access conditions. *Addiction Biology,22*(5), 1160-1168.

Philogene-Khalid, H. L., Hicks, C., Reitz, A. B., Liu-Chen, L., & Rawls, S. M. (2017). Synthetic cathinones and stereochemistry: S enantiomer of mephedrone reduces anxiety- and depressant-like effects in cocaine- or MDPV-abstinent rats. *Drug and Alcohol Dependence,178*, 119-125.

Philogene-Khalid, H. L., Simmons, S. J., Nayak, S., Martorana, R. M., Su, S. H., Caro, Y., . . . Rawls, S. M. (2017). Stereoselective Differences between the Reinforcing and Motivational Effects of Cathinone-Derived 4-Methylmethcathinone (Mephedrone) In Self-Administering Rats. *ACS Chemical Neuroscience,8*(12), 2648-2654.

Schilmoeller, G. L., Schilmoeller, K. J., Etzel, B. C., & LeBlanc, J. M. (1979). Conditional discrimination after errorless and trial-and-error training. *Journal of the Experimental Analysis of Behavior, 31*(3), 405-420.

Seth, P., Scholl, L., Rudd, R. A., & Bacon, S. (2018). Overdose deaths involving opioids, cocaine, and psychostimulants — United States, 2015–2016. *MMWR. Morbidity and Mortality Weekly Report,67*(12), 349-358.

Shanks, K. G., Dahn, T., Behonick, G., & Terrell, A. (2012). Analysis of first and second generation legal highs for synthetic cannabinoids and synthetic stimulants by ultra-performance liquid chromatography and time of flight mass spectrometry. *Journal of Analytical Toxicology,36*(6), 360-371.

Simmons, S. J., Gregg, R. A., Tran, F. H., Mo, L., Weltin, E. V., Barker, D. J., . . . Muschamp, J. W. (2016). Comparing rewarding and reinforcing properties between 'bath salt' 3,4-methylenedioxypyrovalerone (MDPV) and cocaine using ultrasonic vocalizations in rats. *Addiction Biology,23*(1), 102-110.

Smushy. "Wasting a Night on Unknown Chemicals: An Experience with 4-Methylmethcathinone (Mephedrone) (exp81410)". Erowid.org. Mar 26, 2010.

Substance Use and Mental Health Services Administration. (2014). Substance use disorders. Retrieved June 25, 2018, from https://www.samhsa.gov/disorders/substance-use

United States Department of Justice. (2011). The economic impact of illicit drug use on American society. *National Drug Intelligence Center*.

Varner K, Daigle K, Weed P, Lewis P, Mahne S, Sankaranarayanan A, Winsauer P (2013) Comparison of the behavioral and cardiovascular effects of mephedrone with other drugs of abuse in rats. Psychopharmacology 225, 675–685.

Watterson, L. R., Kufahl, P. R., Nemirovsky, N. E., Sewalia, K., Grabenauer, M., Thomas, B. F., . . . Olive, M. F. (2012). Potent rewarding and reinforcing effects of the synthetic cathinone 3,4-methylenedioxypyrovalerone (MDPV). *Addiction Biology,19*(2), 165-174.

Winstock, A. R., Mitcheson, L. R., Deluca, P., Davey, Z., Corazza, O., & Schifano, F. (2010). Mephedrone, new kid for the chop? *Addiction,106*(1), 154-161.

Winstock, A., Mitcheson, L., Ramsey, J., Davies, S., Puchnarewicz, M., & Marsden, J. (2011). Mephedrone: Use, subjective effects and health risks. *Addiction,106*(11), 1991-1996.

Wright, T. H., Cline-Parhamovich, K., Lajoie, D., Parsons, L., Dunn, M., & Ferslew, K. E. (2013). Deaths involving methylenedioxypyrovalerone (MDPV) in upper east Tennessee. *Journal of Forensic Sciences, 58*(6), 1558.

APPENDIX

WESTERN MICHIGAN UNIVERSITY

Institutional Animal Care and Use Committee

ANNUAL REVIEW OF VERTEBRATE ANIMAL USE

RECEIVED
JAN 22 2018
I.A.C.U.C.
Western Michigan University

PROJECT OR COURSE TITLE: Drug Discrimination Studies Of Psychoactive Drugs In Rats
IACUC Protocol Number: 16-02-05
Date of Review Request: 01/19/18 Date of Last Approval: 02/22/17
Purpose of project (select one): ☐Teaching ☒Research ☐Other (specify):

PRINCIPAL INVESTIGATOR OR ADVISOR
Name: Lisa Baker Title: Professor
Department: Psychology Electronic Mail Address: lisa.baker@wmich.edu
CO-PRINCIPAL OR STUDENT INVESTIGATOR
Name: Doug Smith, Trent Bullock, Angela Goolsby, Harmony Risca, Luke Price, Bob Kohler, Rachel Burroughs Title: Students
Department: Psychology Electronic Mail Address:

1. The research, as approved by the IACUC, is completed:
   ☐Yes (Continue with items 4-5 below.) ☒No (Continue with items 2-5 below.)

If the answer to any of the following questions (items 2-4) is "Yes," please provide a detailed explanation on an attached sheet of paper. Include details of any modifications made to the protocol based on new findings or publications, adverse events or mortalities.

2. Have there been any changes in Principal or Co-Principal Investigators? ☐Yes ☒No
3. Have there been any new findings or publications relative to this research that require you to alter your study? ☒Yes ☐No
   Describe the sources used to determine the availability of new findings or publications:
   - ☐No search conducted (Please provide a justification on an attached sheet.)
   - ☐Animal Welfare Information Center (AWIC)
   - ☐Search of literature databases (select all applicable)
     - ☐AGRICOLA ☐Current Research Information Service (CRIS)
     - ☐Biological Abstracts ☒Medline
     - ☐Other (please specify):
     - Date of search: Jan 18, 2018 Years covered by the search: no limits
     - Key words: drug discrimination, synthetic cathinones, MDPV, mephedrone
   - ☒Additional search strategy narrative: discussions with colleagues at research conferences
4. Are there any adverse events, in terms of animal well-being, or mortalities to report as a result of this research? ☒Yes ☐No
   Cumulative number of mortalities: 1
5. Animal usage: Number of animals used during this quarter (3 months): 31 Cumulative number of animals used to date: 60

✒ _____ 1/19/18
Principal Investigator/Faculty Advisor Signature Date

✒ _____
Co-Principal or Student Investigator Signature Date

IACUC REVIEW AND APPROVAL
Upon review of the relevant information regarding this protocol, the IACUC approval for this project has been extended for one year from the date of this signature.

✒ Kathryn A Echer, DVM 2.14.18
IACUC Chair Signature Date