8-2016

Discriminative Stimulus Effects of 3,4-Methylenedioxypyrovalerone and 4-Methylmethcathinone

Michael D. Berquist
Western Michigan University, mdberquist@hotmail.com

DISCRIMINATIVE STIMULUS EFFECTS OF 3,4-METHYLENEDIOXYPYROVALERONE AND 4-METHYLMETHCATHINONE

Michael D. Berquist II, Ph.D.

Western Michigan University, 2016

Recent escalation in the popularity of recreational synthetic cathinone ("bath salts") use has prompted numerous scientific investigations of the neurochemical and behavioral effects of 3,4-methylenedioxypyrovalerone (MDPV) and 4-methylmethcathinone (4-MMC), two of the more common chemical constituents of these illicit "bath salts". Previous neurochemical and electrophysiological studies have revealed that MDPV functions as a blocker, and 4-MMC as a substrate, at monoamine transporters, and both produce transient increases in extracellular monoamines. In addition, previous research has demonstrated that MDPV and 4-MMC support self-administration in nonhuman experimental subjects, and their rewarding effects are observed when paired with contextual cues in nonhuman models of conditioned place preference. Comparatively fewer studies have characterized the discriminative stimulus effects of these drugs using drug discrimination methods. The drug discrimination paradigm is an *in vivo* drug-detection assay with high predictive validity. To further characterize the discriminative stimulus effects of these synthetic cathinones, the current study trained 16 male Sprague-Dawley rats to discriminate either 0.3 mg/kg MDPV ($N = 8$) or 1.0 mg/kg 4-MMC ($N = 8$) from saline.

Once the rats met discrimination acquisition criteria, substitution tests were conducted with compounds that function as dopamine releasers (*d*-amphetamine, (+)-methamphetamine), monoamine transporter inhibitors (MDPV, (-)-cocaine), monoamine transporter releasers (4-MMC, MDMA), a serotonin releaser ((+)-fenfluramine), and an indoleamine hallucinogen (lysergic acid diethylamide, (+)-LSD). Discriminative stimulus control was established in ~35 and ~37 training sessions in the 0.3

mg/kg MDPV group and 1.0 mg/kg 4-MMC group, respectively. In the 0.3 mg/kg MDPV training group, the aforementioned dopamine releasers, monoamine transporter inhibitors, and (+)-fenfluramine produced full substitution, whereas the monoamine transporter releasers and (+)-LSD failed to fully substitute and produced statistical reductions in response rate. In the 1.0 mg/kg 4-MMC training group, all drugs except (+)-LSD and (+)-fenfluramine produced full substitution. Overall, these findings are consistent with human user reports indicating that MDPV and 4-MMC produce interoceptive stimulus effects that are comparable to prototypical drugs of abuse, such as cocaine and MDMA. Future studies with receptor-selective antagonists would be especially valuable to further investigate the neurochemical actions contributing to the discriminative stimulus effects produced by these substances.

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by

Michael D. Berquist II

A dissertation submitted to the Graduate College
in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
Psychology
Western Michigan University
August 2016

Doctoral Committee:

Lisa E. Baker, Ph.D., Chair
Alan D. Poling, Ph.D.
Bradley E. Huitema, Ph.D.
Adam J. Prus, Ph.D.

ACKNOWLEDGEMENTS

Many people contributed to the completion of this dissertation. First, I would like to thank my family, especially my mother Belinda Berquist, father Michael Berquist, and sisters Heidi Berquist-Sharp and Brianna Berquist, for their love and support while completing my graduate studies. I also wish to thank my grandparents, Keith and Deanie LeMoine, and Dale and Elaine Berquist, for teaching me important values. And yes, I've learned that Grandpa Dale is right: "The hardest thing about *anything* is thinking about it." I am a better man because of these people—to everyone, I love you.

Second, I am grateful for the guidance throughout graduate school from my dissertation advisor, Dr. Lisa Baker, and my master's degree advisor, Dr. Adam Prus. I am indebted to my friends Eric Harvey, Zachary Zimmerman, Rachel Burroughs, and Nathyn Thompson for their advice and assistance throughout this study. In addition, I am thankful to the other members of my dissertation committee, Dr. Al Poling and Dr. Brad Huitema, who have helped me become a more skeptical and proficient scientist.

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INTRODUCTION

Drug Use

The consumption of psychoactive substances is a pervasive feature of the human experience. Throughout history, communities worldwide have eaten plants, inhaled vapors, and drank concoctions that produced psychoactive effects. Users consumed many of these substances for particular occasions, such as during spiritual ceremonies (e.g., some evidence suggests that Native Americans may have known of peyote's psychoactive properties for over 5700 years; El-Seedi, De Smet, Beck, Possnert, & Bruhn, 2005) or during divination and healing activities (e.g., coca leaves have a rich history in Peruvian cultures; Valdez, Taboada, & Valdez, 2015). During these occasions, drug effects could have functioned as establishing operations for subsequent behavior (e.g., increasing the reinforcing value of reaching a destination during a hike), or elicited responses that may have been rewarding in their own right to users (e.g., altered perceptual experiences). Despite whatever the behavioral function(s) of the drug may have been, it is clear that many of these communities prospered in their ecological niches and some continue to thrive today regardless of the purity and amount of drug at their disposal.

A different portrayal of drug consumption exists in mainstream, present day American society. In the worst cases, the use and distribution of drugs highlights major media reports with stories of illegal drug trafficking and human tragedies. Docudramatic vignettes are often presented to illustrate how drug consumption had become so severe for users that their lives had all but shattered. Somewhat less ominous, and much less sensationalized, are the millions of Americans who begin each day with a few cups of a caffeinated beverage or a nicotine-packed product. For many of these habitual users, the coffee or cigarette in the morning is commonplace and they can continue drug use without receiving social condemnation (or sympathy). Therefore, it seems a division exists between those who compulsively use drugs to excess, or in an otherwise harmful manner, and those whose use does not disrupt daily functioning to any appreciable extent. A goal of substance use research is to identify the environmental situations in which a person is likely to misuse a drug and form neurochemical and behavioral dependencies.

Characteristics of certain drugs—some defined functionally through interactions with biological/behavioral systems and others socially through cultural norms—may pose greater risks to users' welfare. Such characteristics may include a drug's dose-dependent, unconditioned physiological effects

(e.g., endorphin-releasing effects in the central nervous system), its legal status or social acceptance, and its behavioral function(s) for the user (e.g., ethyl alcohol can produce increases in sociability among users). It is noteworthy to emphasize that some individuals often consider drugs as *inherently* "good" or "bad", although it is likely that many of these assessments are due to socio-political factors or correlations between their use and some adverse event, rather than scientific evidence demonstrating that a drug produces positive or untoward effects. For example, in 2014, 22.2 million people were reported to use marijuana for nonmedical purposes (Center for Behavioral Health Statistics and Quality, 2015). This figure may seem alarming, but it is presently unclear whether marijuana use is strongly associated with relatively high levels of social or personal harm. Taylor et al. (2012) surveyed 292 clinical experts across Scotland to evaluate the relative harm to self and others of 19 commonly used drugs. These authors found that heroin, crack cocaine, crystal methamphetamine, alcohol, and cocaine were ranked as the top five, most harmful drugs in a combined personal-social harm measure. Contrariwise, lysergic acid diethylamide (LSD), Ecstasy/MDTA, methylphenidate/Ritalin, psilocybin ("magic mushrooms"), and cannabis were ranked as the five least harmful drugs. In addition, the clinical experts ranked nicotine as the seventh most harmful drug on this list. Although these now dated findings may only generalize to Scottish individuals (also see Nutt, King, & Phillips, 2010), it is possible (though, to the present author's knowledge undetermined) that American clinical experts would report similar relative indices of harm for these drugs in the United States. Indeed, during the time of this writing, cannabis and its major psychoactive constituent, Δ9-tetrahydrocannibol, is becoming legal for recreational possession and use in some states of the U.S.; although, according to U.S. federal regulations, the drug is illegal to use or possess.

In contrast to the aforementioned use of marijuana in the United States in 2014, daily consumption of a caffeinated beverage was as high as 85% of the total U.S. population (Mitchell, Knight, Hockenberry, Teplansky, & Hartman, 2014). Perhaps in the future a similar percentage of the U.S. population will be consuming marijuana or other drugs that are currently labeled as illicit. Historical accounts have demonstrated that the popularity of some drugs wax and wane over time and cultures, leading to social acceptance for some substances (e.g., cannabis, nicotine, ethyl alcohol), but not others (e.g., heroin, methamphetamine, LSD). Certain drugs in one particular chemical class, the synthetic cathinones (referred to as "bath salts"), are currently considered an international public concern because recent media, legal, and

toxicology reports have identified associations between consumption of these drugs and adverse events (e.g., death, crimes). Previous experimental reports have revealed that particular substances in the class of synthetic cathinones function as potent reinforcers for appetitive responses and possess relatively high abuse potential as inferred using common methods in preclinical research (see below). More experimental research on these substances is necessary to further characterize their behavioral effects. The research included herein provides insight into the possible interoceptive effects produced by two synthetic cathinones that may assist in informing individuals in the public, private, and government sectors of the drugs' effects on behavior.

Consistent with the foregoing introduction, it remains undetermined if the next emergent class of drugs will replace the consumption of the synthetic cathinones, whether societies will become more liberal concerning synthetic cathinone use, or, if these substances remain labeled illicit, perhaps their popularity will resurge in the future. In any event, rigorous experimental assessments of these drugs may prove valuable for future efforts devoted toward limiting, reducing, or simply knowing the effects produced following their consumption. The present dissertation includes an investigation of the discriminative stimulus effects of two synthetic cathinones, 4-methylmethcathinone (4-MMC) and 3,4-methylenedioxypyrovalerone (MDPV), using drug discrimination procedures in male Sprague-Dawley rats. Before discussing an overview of the synthetic cathinones, a brief review of the parent compound, cathinone, is presented.

Cathinone

Use, prevalence, and pharmacokinetics. Cathinone [(*S*)-(-)-α-aminopropiophenone] is an alkaloid found within the leaves of the *Catha edulis* Forsk shrub. The plant is native to regions of Africa and the Arabian Peninsula where for centuries indigenous communities have consumed its leaves as an "energizer" for work and climbing activities (Kennedy, Teague, Rokaw, & Cooney, 1983), during social settings (Kalix, 1988), and to experience psychostimulant-like effects (for review, Pantelis, Hindler, & Taylor, 1989). Other names for *Catha edulis* Forsk, each of which is region-specific, include khat (most common), qat, chat, mirra, or qaad/jaad (Alem, Kebede, & Kullgren, 1999).

Khat use is common among individuals from Yemen and other East African cultures (European Monitoring Centre for Drugs and Drug Addiction [EMCDDA], 2015a). Khat users commonly chew the

plant's leaves, a process which extracts ~90% of available cathinone from their contents (Toennes & Kauert, 2002; Toennes, Harder, Schramm, Niess, & Kauert, 2003); although, previous reports have indicated that dried khat leaves are sometimes brewed in teas or smoked (Hodgkinson, 1962; Giannini, Miller, & Turner 1992). While chewing the plant, cathinone is absorbed through the user's buccal mucosa and gastrointestinal tracts, and it reaches peak plasma concentrations about two hours post-consumption (Toennes, Harder, Schramm, Niess, & Kauert, 2003). Metabolism of cathinone to norephedrine (or phenylpropanolamine) and norpseudoephedrine (or cathine) occurs following ingestion (Brenneisen, Geisshüsler, & Schorno, 1986) and cathinone can be detected in urine for 22-26 hours post-consumption (Toennes & Kauert, 2002). For an extensive review of khat's chemical constituents and pharmacokinetics, see Feyissa and Kelly (2008).

Legal status. Despite khat's (and cathinone's) popularity among recreational users in Eastern Africa and Arabian regions, several countries have created legal regulations to control its use and distribution. Indeed, cathinone was listed as Schedule I at the United Nations Convention on Psychotropic Substances (United Nations Office on Drugs and Crime, 2016). In addition, the European Union has classified khat as a controlled substance in 15 of its 27 member countries (EMCDDA, 2011), and the United States placed cathinone on the list of controlled substances in 1993 (Drug Enforcement Agency (DEA), 1993). In Canada, cathinone is a Schedule III substance under the 1996 Controlled Drugs and Substances Act (Government of Canada, 2016). Although khat use and possession became illegal in the U.S. in 1993, law enforcement agencies have seized large quantities of the plant in the years following. For example, according to the Federal-wide Drug Seizure System, legal authorities seized 89,669 kilograms of khat in 2010 (DEA, 2013). Further, the DEA (2013) reported that distributors from Africa and the Middle East were responsible for transporting khat into the United States. It is noteworthy that synthetically-derived formulations of cathinone (viz. synthetic cathinones) may have replaced khat availability among the illicit drug markets (for review, Nichols, Khondkar, & Gibbons, 2015).

Neurochemical profile. Comprehensive characterization of a drug's neurochemical effects serves as a useful starting point for understanding how and why it affects behavior. One fruitful area of research devoted toward investigating how a drug's molecular structure affects its pharmacological activity is through structure-activity relationship studies (for review, Glennon and Young, 2011a). Essentially, some

chemical property of a drug is altered or isolated (e.g., optical rotation, chirality) and its pharmacological activity is subsequently assessed. Among the many sub-areas within the study of structure-activity relationships, investigations of drug enantiomers and optical isomers (viz. stereochemistry) are especially valuable. For example, Dal Cason (unpublished observations, cited in Sparago et al. 1996) reported that the *sinister* enantiomer (usually denoted by an *S* preceding a drug's name[1]) of methcathinone, a cathinone derivative, was more commonly found in the illicit drug market than the *R* enantiomer. Such information can be useful for determining whether to evaluate the effects a drug enantiomer or a racemic drug using experimental methods, and, as in the foregoing case of methcathinone, useful for distinguishing if an enantiomer is more potent at producing one effect (e.g., locomotor stimulant effects) over another (e.g., neurotoxicity). In any case, evaluations of a drug's structure-activity relationships has been and continues to be an active area in drug use research.

Previous studies have assessed the neurochemical and behavioral effects of racemic cathinone or cathinone enantiomers using established methods in preclinical research. For example, Glennon and Liebowitz (1982) demonstrated that the *S*(-)-cathinone enantiomer possesses twice the affinity as racemic (±)-cathinone for serotonin receptors (measured using p*A*2 values obtained from Schild plots; see Arunlakshana & Schild, 1959). These receptor affinity values were measured using rat fundus preparations. The authors of that study were among the first to evaluate the structure-activity relationship of a cathinone enantiomer. In a later study, Rothman et al. 2003 demonstrated that *S*(-)-cathinone has relatively potent releasing effects of norepinephrine and dopamine, as measured in cloned human substrates (see cathinone as test agent at Psychoactive Drug Screening Program: K_i Database, n.d.). The norepinephrine- and dopamine-releasing effects of *S*(-)-cathinone are similar to *d*-amphetamine (e.g., Rothman et al. 2001). Overall, the foregoing studies have demonstrated the relative potency of a cathinone enantiomer compared to racemic cathinone (Glennon & Liebowitz, 1982) and the neurochemical targets to which *S*(-)-cathinone produces potent, monoamine releasing effects (Rothman et al. 2003). These results are in part valuable for determining the neurochemical bases of cathinone's behavioral effects.

[1] A racemic drug (or racemate) consists of equal amounts of left- and right-handed enantiomers and is sometimes abbreviated as (±) or (*SR*), which precedes the drug's name. A *rectus* enantiomer is often denoted by *R* preceding the drug name.

In addition to the aforementioned *in vitro* experiments that have evaluated cathinone's neurochemical effects, previous studies have included *ex vivo* techniques as well. For example, Fleckenstein et al. (1999) examined the extent to which cathinone would prevent (i.e., compete with) [^{3}H]dopamine and [^{3}H]serotonin uptake at dopamine (DAT) and serotonin transporters (SERT) in rat striatal synaptosomes, respectively. Groups of rats had previously received a single injection or multiple injections (sc) (i.e., four injections spaced two hours apart) of 40 mg/kg cathinone and were decapitated one hour after the final injection. Compared to saline-treated rats, single or multiple injections of 40 mg/kg cathinone produced statistically significant reductions in dopamine uptake at DAT, and multiple injections produced significant reductions in serotonin uptake at SERT. Overall, these data demonstrate that 40 mg/kg cathinone reduces the transporter functions of DAT, and, in a dosing-specific manner, SERT. Further, in discussing the singular effect of cathinone (and other psychoactive substances not mentioned here) on SERT functioning observed only after the multiple injection procedure, Fleckenstein et al. suggested that *in vitro* characterizations (or single drug administrations) may not accurately portray a drug's effects when subjects are repeatedly exposed to drugs *in vivo*.

The foregoing studies illustrate how different approaches can be used to determine a drug's neurochemical effects. Though beyond the scope of the present document to further discuss cathinone's neurochemical actions (for review, Feyissa & Kelly, 2008), it is clear that experiments including *in vitro* and *ex vivo* techniques can serve as useful starting points for subsequent investigations. Moreover, such techniques make available comparisons of drugs with imperfectly known neurochemical effects, such as cathinone during the time at which these studies were conducted, to prototypical psychoactive drugs, such as amphetamine.

Psychopharmacology of cathinone. Researchers have extensively investigated the bio-behavioral effects of cathinone using several measures commonly used in human psychopharmacology research. For example, Brenneisen, Fisch, Koelbing, Geisshüsler, and Kalix (1990) observed in human volunteers that cathinone consumption produces increases in blood pressure, heart rate, psychostimulant-like, and euphorigenic effects—some of which are consistent with the habitual users' reports mentioned in the introduction of the present document. Previous studies have reported that compared to non-khat users, habitual khat users display irregular diurnal salivary cortisol levels and blunted blood pressure levels in

response to mistakes made on a mental arithmetic task (al'Absi et al., 2013), poorer accuracy and increased reaction times in working memory and task-switching procedures, respectively (Colzato, Ruiz, van den Wildenberg, & Hommel, 2011), and disrupted sleep (Nakajima et al., 2014). Amid these findings, Numan (2004) failed to observe high incidents of psychological morbidity in a sample of 800 Yemini adults (age range: 15-76) of which over 80% of males and 40% of females indicated at least a single episode of khat use in their lifetimes. Therefore, similar to the untoward effects produced by the consumption of other illicit drugs, khat's adverse effects are likely dependent on interactions with other environmental and biological factors present during the time of consumption.

Reports of cathinone's effects on nonhuman animal behavior using common procedures in behavioral pharmacology research are also widespread. Select studies highlighting the behavioral effects of cathinone will now be presented for exposition. Gugelmann, von Allmen, Brenneisen, and Porzig (1985) compared the effects of (+)- and (-)-cathinone optical isomers[2] to (+)-amphetamine on locomotor activity in rats. Among the doses tested, 3.0 and 6.0 mg/kg (-)-cathinone produced greater locomotor activity than (+)-cathinone, and greater levels than 6.0 mg/kg (+)-amphetamine. Johanson and Schuster (1981) demonstrated in rhesus monkeys that *dl*-cathinone and *l*-cathinone decreases food-reinforced responding and maintains self-administration. In a later study, Woolverton and Johanson (1984) reported that in rhesus monkeys trained to self-administer cocaine or *dl*-cathinone under a drug-drug, discrete-trials choice procedure, increasing doses of cocaine were necessary to shift a preference to cocaine from *dl*-cathinone as the dose of *dl*-cathinone increased. Moreover, these authors found that the drug-drug choice results obtained from the foregoing procedure revealed that the reinforcing efficacies of *dl*-cathinone and cocaine were comparable.

Of particular relevance to the experiment described herein, previous reports have also investigated cathinone's discriminative stimulus effects using drug discrimination procedures. Drug discrimination procedures typically involve training experimental subjects (usually rodents) to discriminate between a

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[2] In the context of drug molecules, "optical isomers" refers to the direction in which a drug rotates when exposed to polarized light. If the isomer rotates clockwise in the direction of the oncoming light stimulus, this is referred to as *dextrorotary* (also referred to as *d* or (+) preceding a drug name). Contrariwise, if the isomer rotates counter-clockwise in the direction of the oncoming light stimulus, this is referred to as *levorotary* (also referred to as *l* or (-) preceding a drug name). Racemic mixtures of optical isomers can be referred to as *dl*, (±), or "nothing" preceding a drug name.

dose of a psychoactive substance (e.g., injection of 10 mg/kg cocaine; the "training drug") from its absence (e.g., injection of saline). Specifically, subjects are differentially reinforced (e.g., using a food pellet as a reinforcer) to emit one response in the presence of the drug (e.g., lever press on left lever in operant conditioning chamber) and emit a different response in the absence of the drug (e.g., right lever press). Once subjects reach some specified level of discrimination accuracy (e.g., >80% condition-appropriate responding for 8 out of 10 consecutive training sessions), then other doses of the training drug can be tested for substitution (i.e., testing whether the interoceptive cue produced by different doses of the training drug or doses of a different drug "substitute" for the training drug cue). Substitution generally falls into three qualitative categories: no substitution, partial substitution, or full substitution. No substitution is generally considered ≤20% drug-lever selection, partial substitution is >20% but <80% drug-lever selection, and full substitution is ≥80% drug-lever selection. Thus, for example, if a test drug produces full substitution, then it is declared that the test drug and training drug produce similar discriminative stimulus effects. See below for additional information regarding the drug discrimination paradigm.

Schechter, Rosecrans, and Glennon (1984) reported the first known experiment to train ARS/Sprague-Dawley rats to discriminate (±)-cathinone (0.6 mg/kg) from saline. All rats acquired the discrimination (i.e., met the authors' criterion of 8 out of 10 consecutive sessions of condition-appropriate responding) within 30 sessions. Injections of 0.8 and 0.4 mg/kg *d*-amphetamine equipotently (cathinone ED_{50} value: 0.21 mg/kg; *d*-amphetamine ED_{50} value: 0.24 mg/kg) produced full substitution in the 0.6 mg/kg cathinone-trained rats, suggesting common discriminative stimulus properties between the two drugs. In contrast, apomorphine (0.16, 0.24, and 0.32 mg/kg) failed to produce full substitution. Schechter, Rosecrans, and Glennon (1984) concluded that the similarity in potency and responding between (±)-cathinone and *d*-amphetamine suggests that the drugs may produce their discriminative stimulus effects through a common pharmacological mechanism of action.

In a later study, Goudie, Atkinson, and West (1986) investigated whether doses of several centrally-acting stimulants (i.e., *d*-amphetamine, cocaine, methylphenidate, pipradrol, and cathine) would generalize in rats trained to discriminate 2.0 mg/kg *dl*-cathinone from saline. The median sessions required to reach the discrimination criterion (i.e., 10 consecutive sessions of responding on the injection-appropriate lever prior to the delivery of the first reinforcer) was 21 sessions. All of the foregoing

stimulants produced full substitution (i.e, in a dose-dependent manner, suggesting that these stimulants share common centrally-acting interoceptive effects. Moreover, the sympathomimetic, *p*-hydroxyamphetamine, failed to fully substitute in the rats, indicating that 2.0 mg/kg cathinone appears to produce its interoceptive effects through a centrally-mediated cue. Stimulus blockade tests with a D_2 receptor antagonist, haloperidol, were also performed in this experiment. Pre-treatment with 0.25 or 0.3 mg/kg haloperidol in conjunction with 2.0 mg/kg cathinone reduced percent cathinone-lever selection from 85.7% (i.e., the mean percent selection value of 2.0 mg/kg cathinone) to 66.7% and 50%, respectively. Because complete blockade was not produced by the largest dose of haloperidol tested (0.3 mg/kg), other receptor actions are likely involved in mediating the 2.0 mg/kg cathinone cue (Goudie, Atkinson, & West, 1986).

To further investigate the discriminative stimulus effects of cathinone enantiomers, previous studies have evaluated whether these compounds generalize in groups of rats trained to discriminate a hallucinogen or a centrally-acting stimulant from saline. For example, Glennon (1986) investigated the discriminative stimulus effects of (±)-, *S*(-), and *R*(+)-cathinone in rats trained to discriminate 1.0 mg/kg (±)-DOM (2,5-dimethoxy-4-methylanalog) or 1.0 mg/kg (+)-amphetamine sulfate from saline. The results of this study revealed that rats injected with doses of the cathinone enantiomers (0.2-20 mg/kg) failed to show substitution in DOM-trained rats, but all enantiomers produced full generalization in the amphetamine-trained rats (ED_{50} values of cathinone enantiomers expressed in mg/kg: (±)- 0.72, *S*(-) 0.34, and *R*(-)4.41). Based on these obtained ED_{50} values, Glennon (1986) concluded that the *S*(-) cathinone enantiomer is approximately ten times more potent as the *R*(-) enantiomer in producing amphetamine-lever responding (i.e., amphetamine-like interoceptive effects).

A later study by Young and Glennon (1998) evaluated whether racemic cathinone and the *S*-cathinone enantiomer would substitute in rats trained to discriminate the cathinone derivative methcathinone from saline. In this study, rats were trained to discriminate 0.5 mg/kg *S*(-)-methcathinone from saline under a variable-interval 15-sec schedule of sweetened milk reinforcement. Doses of (±)-cathinone (0.25, 0.50, 0.85, and 1.0 mg/kg) and *S*(-)-cathinone (0.125, 0.20, 0.25, and 0.50 mg/kg) were among the compounds tested for stimulus substitution. Racemic cathinone and *S*-cathinone produced full substitution in the *S*(-)-methcathinone-trained rats, and the ED_{50} value of the *S*(-)-cathinone isomer (0.19

mg/kg) was reportedly lower than the ED_{50} value of (±)-cathinone (0.41 mg/kg), although this difference was not statistically significant (i.e., the associated 95% confidence intervals did not overlap). Based on these findings, Glennon and Young (1998) concluded that the *S*(-)-methcathinone enantiomer may produce its discriminative stimulus effects through dopaminergic mechanisms, similar to the effects of cathinone.

The foregoing drug discrimination experiments using racemic cathinone or a cathinone enantiomer as the training drug, or testing whether cathinone enantiomers would substitute in subjects trained on some other centrally-acting compound, supports the use of drug discrimination methods to investigate quantitative and qualitative effects of cathinone *in vivo*. Though beyond the scope of the present document to describe every drug discrimination study involving cathinone, the foregoing studies demonstrated that drugs that enhance dopamine release or otherwise increase extracellular dopamine levels (e.g., amphetamine, cocaine, methylphenidate) produce discriminative stimulus effects that are comparable to cathinone (e.g., Schechter, Rosecrans, & Glennon,1984; Goudie, Atkinson, & West, 1986), and that stimulation of the D_2 receptor in particular mediates some aspect of cathinone's interoceptive effects (Goudie, Atkinson, & West, 1986). Contrariwise, drugs that stimulate serotonin receptors, such as DOM, do not appear to produce discriminative stimulus effects that are similar to cathinone (e.g., Glennon, 1986).

The foregoing summary of the behavioral and neurochemical effects of cathinone demonstrates the utility of experimentation in preclinical research. At the time the cathinone research was conducted, investigations of the abuse liability and pharmacological effects of this drug was a socially-relevant endeavor. Pertinent to the experiment planned herein, the discussion will now turn to synthetic analogues of cathinone and the variety of experimental procedures in which they have been tested.

Synthetic Cathinones

Description, use, prevalence. Recent research interests in studying the behavioral and neurochemical effects of substances within the aforementioned class of drugs known as the "synthetic cathinones" ("bath salts", "plant food") are extensive. These drugs, as their class label implies, derive from the constituent β-ketoamphetamine structure of cathinone. Some synthetic cathinones (i.e., β-ketos) are used for medicinal purposes, such as diethylpropion (Tenuate®; produces anorexigenic effects, used to treat obesity) and bupropion (Wellbutrin®; used as antidepressant and for smoking cessation). Among the illicitly-labeled synthetic cathinones are 3,4-methylenedioxypyrovalerone (MDPV),

4-methylmethcathinone (4-MMC, mephedrone), methcathinone (epedrone), methylone (βk-MDMA, 3,4-methylenedioxy-N-methylcathinone), methedrone (βk-PMMA, 4-methoxymethcathinone), and α-pyrrolidinopropiophenone (EMCDDA, 2015b). It is noteworthy that there are other derivatives in addition to those mentioned above, some of which are used medicinally and others that are labeled illicit (see EMCDDA, 2015b).

According to the UNODC World Drug Report (2015), synthetic cathinones were the third fastest growing (15 percent growth) new psychoactive substances worldwide in 2014 (third to synthetic cannabinoids at 39 percent and phenethylamines at 18 percent). Common routes of administration among synthetic cathinone users include swallowing, insufflation, inhalation, or injection (National Institute on Drug Abuse, 2016). According to Erowid (2015a;b), oral doses of MDPV range from 2-25 mg and 4-MMC range 15–300+ mg. A recent study revealed that in a sample of 145 4-MMC users in Sweden, 84.4% insufflated and 11% injected, and those who injected used 4-MMC more frequently and at higher doses (Kapitány-Fövény et al., 2015). In this same sample, the injector sub-group also comprised 37.5% opiate users, suggesting that individuals who inject synthetic cathinones are likely to consume other drugs that are delivered via similar drug administration routes. Further research is necessary to identify if this is an ecologically-relevant issue among synthetic cathinone users.

Toxicities following the consumption of synthetic cathinones were well publicized by widespread media attention in the early 2010s. Previous media reports have documented cases of violent and bizarre behaviors, and death (e.g., Whitney, 2011; Campbell, 2012; "Police: Man on", 2013). Due in part to these reports, the Drug Enforcement Agency (DEA, 2011) added MDPV, 4-MMC, and methylone to the Schedule I list of controlled substances on October 21, 2011. Moreover, an additional 10 bath salt constituents were labeled as Schedule I March 7, 2014 (DEA, 2014). Despite these legal ramifications, previous reports suggest that synthetic cathinone users may have continued to acquire these substances using the Internet or other covert tactics (e.g., Schifano et al., 2011; for review, Vardakou, Pistos, & Spiliopoulou, 2011). In 2011, consumption of synthetic cathinones accounted for 22,904 emergency room visits and over 50% (~11,987 of cases) of the visits involved bath salts consumed in combination with other drugs (The DAWN report, 2013). Other sources indicate that from January 1, 2016, to May 31, 2016, 166 human exposures to bath salts were reported to poison control centers in the U.S. (American Association of

Poison Control Centers, n.d.), which included incidents of seizures, violent behavior, and hallucinations. Further, the DEA (2011) revealed in 2011 that law enforcement agencies most commonly encountered two constituents, MDPV and 4-MMC. As such, researchers have devoted considerable attention to the neurochemical and behavioral effects of MDPV and 4-MMC.

Previous Research on MDPV and 4-MMC

The current literature corpus on MDPV and 4-MMC includes a broad range of research findings. Researchers have evaluated the neurochemical and behavioral effects of MDPV and 4-MMC using a variety of experimental methods commonly used in preclinical substance abuse research. Several of the research findings are presented below for exposition.

Electrophysiological and neurochemical effects. Recent electrophysiological reports (Cameron, Kolanos, Solis, Glennon, & De Felice, 2013; Cameron, Kolanos, Vekariya, De Felice, & Glennon, 2013) demonstrated that MDPV produces an outward hyperpolarizing current at the human dopamine transporter (hDAT), similar to the effects of cocaine. In addition to possessing a strong binding affinity to DAT (e.g., Simmler et al., 2013), studies have revealed that MDPV also possesses relatively strong binding affinity for the norepinephrine transporter (NET) (Simmler et al., 2013), but exerts comparatively weaker effects (i.e., less binding affinity) on serotonin transporter (SERT) reuptake (Baumann et al., 2013a; Simmler et al., 2013; Eshleman, Wolfrum, Hatfield, Johnson, Murphy, & Janowsky, 2013). Moreover, MDPV functions as an inhibitor at hDAT and NET *in vitro* and it produces dose-dependent increases in extracellular dopamine in rat nucleus accumbens *in vivo* (Baumann et al., 2013). In a recent study, Kolanos et al. (2015) demonstrated that *S*(+)-MDPV was more potent than racemic MDPV or *R*(-)-MDPV in blocking DAT and NET functioning, however no differences were observed among the isomers in inhibiting SERT functioning.

Contrary to the foregoing effects of MDPV at hDAT, 4-MMC produces an inward depolarizing current at hDAT (Cameron, Kolanos, Solis, Glennon, & De Felice, 2013; Cameron, Kolanos, Vekariya, De Felice, & Glennon, 2013), similar to the effects of methamphetamine. Current research has also demonstrated that 4-MMC is a transporter substrate at NET, DAT, and SERT, similar to the pharmacological effects of 3,4-methylenedioxymethamphetamine (MDMA or Ecstasy) (Baumann et al., 2012); although, 4-MMC has a comparatively stronger binding affinity to DAT. Indeed, Kehr et al. (2011)

found that injections (sc) of 3.0 mg/kg 4-MMC in rats produced time-dependent increases in extracellular dopamine levels in the nucleus accumbens (peak at 496% of control), as determined using microdialysis procedures. In that study, 3.0 mg/kg 4-MMC also produced statistically greater levels of dopamine and serotonin in the rats when compared to saline-treated controls. In addition to these findings, Simmler et al. (2013) demonstrated that 4-MMC also possesses relatively strong binding affinity (i.e., K_i: 2.1 ± 0.7) for serotonin 5-HT_{2A} receptors. Further, Eshleman et al. (2013) observed that 4-MMC functions as an antagonist of inositol-1-phosphate formation at serotonin h5-HT_{2A} receptors, indicating that this drug elicits intracellular events that may further modulate neural activity. For a current review of MDPV and 4-MMC's pharmacological properties, see Valente, Guedes de Pinho, de Lourdes Bastos, Carvalho, and Carvalho (2014).

In addition to studies that have examined the acute pharmacological effects of MDPV and 4-MMC at receptor targets in the central nervous system, other reports have demonstrated that these drugs produce effects on peripherally-located targets (i.e., outside the CNS) as well. For example, Araújo et al. (2015) demonstrated that MDPV produces cytotoxicity in rat hepatocytes, similar to the effects of MDMA. Shortall, Green, Swift, Fone, and King (2012a), found that 10 mg/kg 4-MMC produced a decrease in tail and rectal temperature in adult male Lister hooded rats. Similar hypothermic effects of 4-MMC have been demonstrated elsewhere (e.g., Shortall et al., 2012b; Wright et al., 2012; Miller et al., 2013). Contrary to 4-MMC, MDPV produces dose- and ambient-temperature-dependent hyperthermic effects measured using peripherally-located telemetry devices in mice (e.g., Fantegrossi, Gannon, Zimmerman, & Rice, 2013). Kiyatkin, Kim, Wakabayashi, Baumann, and Shaman (2015) observed similar effects, with MDPV producing dose-dependent increases in brain temperature. Given that all drugs produce multiple effects at the behavioral level or physiological level of analysis, such findings highlight the need of evaluating drug effects in targets beyond the central nervous system.

Polysubstance use in humans. Although not directly relevant to the experiment described herein, it is noteworthy that polysubstance use is commonplace in the consumption of illicit or legal drugs. In some cases, simultaneous use of multiple drugs may pose greater health and safety risks to users given the combined actions of the drugs. A recent study observed that among a sample of 30 deceased individuals aged 16-24 years old in the United Kingdom who used 4-MMC, 63% died due to accidental poisoning and

87% used 4-MMC with other drugs (Loi et al., 2015). It may be that users combine other drugs with the synthetic cathinones to enhance their stimulant and euphoric effects. Previous reports on human consumption of MDPV and 4-MMC indicated that these substances produce interoceptive stimulus effects similar to other illicit drugs, such as cocaine and MDMA (Winstock et al., 2011a;b; for review, Ross, Reisfield, Watson, Chronister, & Goldberger, 2012), however, further research is necessary to understand if drug combinations lead to a desired increase in subjective effects.

Regardless of whether users achieve the desired combined effects of mixing bath salts with other drugs, or if even they consume mixtures knowingly, recent toxicology reports have documented cases in which human users simultaneously displayed levels of synthetic cathinones and other drugs present in bodily fluids (e.g., Wyman et al., 2013; Murray, Murphy, & Beuhler, 2012; Aromatario, Bottoni, Santoni, & Ciallella, 2012; Lusthof et al., 2011). These findings indicate that polysubstance use may be common among synthetic cathinone users, although, as mentioned, further research is necessary to confirm if the drugs are knowingly consumed together, or if users acquire the drugs already mixed. In any event, non-human research indicates that simultaneous exposure to bath salt constituents with other drugs may pose greater health risks to users, including enhanced neurotoxicity. For example, recent studies have demonstrated in mice that pretreatment with 4-MMC enhanced dopamine nerve ending toxicity produced by methamphetamine, amphetamine, or MDMA (Angoa-Pérez et al., 2013); however, in a later study (Angoa-Pérez, Kane, Herrera-Mundo, Francescutti, & Kuhn, 2014), 4-MMC pretreatment appeared to produce no effect on the health of serotonin nerve endings. Contrariwise, Anneken, Angoa-Pérez, and Kuhn (2015) observed that MDPV pretreatment in mice attenuated methamphetamine-induced dopamine nerve ending neurotoxicity. The authors of that study suggested that substrates for DAT (e.g. 4-MMC) appear to enhance methamphetamine neurotoxicity, but blockers at DAT (e.g., MDPV) may have a neuroprotective effect. Further research is necessary to dissociate the relative effects of monoamine transporter blockers from substrates in terms of pharmacologically-induced neurotoxicity. In any case, it remains to be determined if early exposure to MDPV or 4-MMC produces similar effects in recreational human drug users. Future studies examining the effects of ecologically-relevant drug mixtures on biological and behavioral measures seems warranted.

Locomotor effects and ambulatory sensitization. It is common in behavioral pharmacology research to evaluate drug effects on ambulatory measures in non-human models. Some researchers have suggested that all drugs producing locomotor-stimulant effects possess addiction potential, largely because such drugs stimulate dopaminergic pathways that are associated with "approach behaviors" (see Wise & Bozarth, 1987). Nevertheless, current views submit that changes in dopaminergic activity (e.g., increases in dopamine efflux) are not common to all misused drugs (for review, Nutt, Lingford-Hughes, Erritzoe, & Stokes, 2015), and therefore, current research efforts should consider a broader spectrum of drug-receptor-pathway mechanisms responsible for drug misuse and chemical dependencies. Consistent with traditional precedence, recent studies have examined the locomotor effects of MDPV and 4-MMC. Several of these experiments are reported below.

Shortall et al. (2013) injected rats (ip) with 1 or 4 mg/kg (-)-cathinone 1, 4, or 10 mg/kg (±)-4-MMC-HCl, 10 mg/kg (±)-MDMA-HCl, or saline and measured locomotor activity for 60 min. The results revealed that rats injected with 10 mg/kg 4-MMC, 10 mg/kg MDMA, and 1 or 4 mg/kg cathinone displayed statistically greater levels of activity than saline-treated rats. Lisek et al. (2012) demonstrated that pretreatment with a dopamine D_1 receptor antagonist (SCH 23390; 0.5 mg/kg) attenuated the acute locomotor stimulant effects of 5 mg/kg 4-MMC, and pretreatment with a dopamine D_2 receptor antagonist (sulpiride; 40 mg/kg) enhanced the acute effects of 5 mg/kg 4-MMC in male Sprague-Dawley rats. MDPV is comparatively more potent than 4-MMC at producing locomotor-stimulant effects in rodents. For example, Gatch, Taylor, and Forster (2013) demonstrated that mice injected with MDPV (1, 3, or 10 mg/kg) displayed statistically greater, time-dependent levels of ambulation counts compared to saline-treated mice, whereas in the same study, only 3 or 10 mg/kg 4-MMC produced greater levels of ambulation counts than saline-treated mice; although, it is worth noting that the ED_{50} values for locomotor activity did not differ between the MDPV-treated- (1.26 ± 0.08; expressed in mg/kg as mean ± *SE*) and 4-MMC-treated mice (1.38 ± 1.22). Aarde, Huang, Creehan, Dickerson, and Taffe (2013a) demonstrated that male Wistar rats injected (sc) with 0.5 or 1.0 mg/kg MDPV displayed statistically greater, time-dependent increases in locomotor activity counts compared to saline-treated rats. For an excellent review of the locomotor effects of 4-MMC and MDPV in rodents, see Gregg and Rawls (2014).

In addition to evaluations of the acute locomotor effects of MDPV and 4-MMC, other research has assessed the effects of repeated exposure to these drugs on ambulatory responses. If a subject's ambulatory responses progressively increase as function of repeated and intermittent drug exposure, this phenomenon is termed "behavioral sensitization". Evidence of drug-induced behavioral sensitization is suggested to reflect neuroadaptive changes in brain areas that are implicated in compulsive drug use and forming chemical dependencies (Pierce & Kalivas, 1997; Robinson & Berridge, 2001), and involve physiological pathways that are affected by prototypical psychostimulants (Vanderschuren & Kalivas, 2000). It is worth nothing that the *behavioral* data recorded using sensitization procedures seems most applicable for evaluating if repeated drug exposure produces progressive increases in drug sensitivity (*cf*. tolerance). Such drug sensitivity is subject to a variety of procedural variables (e.g., drug exposure and activity recording occurs in the same context instead of a different context; see Ohmori, Abekawa, Ito, & Koyama, 2000; Phillips, Pastor, Scibelli, Reed, & Tarragón, 2011). Accordingly, behavioral sensitization procedures are commonly used in preclinical research to characterize the behavioral effects of psychoactive substances under specific experimental conditions.

Recent studies have demonstrated 4-MMC-induced ambulatory sensitization in mice (Lisek et al., 2012; Berquist, Peet, & Baker, 2015) and rats (Gregg, Tallarida, Reitz, McCurdy, & Rawls, 2013). In addition, Gregg, Tallarida, Reitz, and Rawls (2013) demonstrated that 4-MMC cross-sensitizes to the stimulant effects of cocaine in Sprague-Dawley rats. At the time of this writing, Berquist and Baker (unpublished findings) and Berquist, Traxler, Mahler, and Baker (2016) completed several studies that tested doses of MDPV or 4-MMC using locomotor sensitization procedures. The studies conducted at Western Michigan University were not completed to evaluate the effects of MDPV or 4-MMC on levels of locomotor activity per se, but rather to assess if these drugs enhanced (or suppressed) the locomotor effects of other drugs (e.g., cocaine) when combined in mixtures. Based on these studies, adult male Sprague-Dawley rats injected with 0.5 mg/kg MDPV displayed within-group sensitization (i.e., day 7 horizontal activity was statistically greater than day 1 activity following a seven-day consecutive, daily-dosing procedure), rats previously injected with select mixtures of MDPV and 4-MMC displayed statistically greater, time-dependent levels of horizontal activity following an acute injection of cocaine (5 mg/kg) compared to rats previously injected with saline (Berquist, Traxler, Mahler, & Baker, 2016), and

there is some evidence collected in-house suggesting that in mice, there may be differences in sensitivity to the locomotor stimulant effects of mixtures of MDPV and 4-MMC depending on subject sex. Based on these findings, and other studies completed at Western Michigan University (manuscripts in preparation), it is predicted that the locomotor sensitization assay will serve as a useful model of exploring the behavioral effects of drug mixtures.

Models of reinforcement and reward. Procedures for evaluating the reinforcing or rewarding effects of drugs include response-dependent (e.g., intravenous self-administration) and response-independent components. Nonhuman experimental subjects are most often used in behavioral pharmacology research given practical and ethical restraints. It is important to recognize that with each of the models, laboratory control over extraneous (but perhaps socially or clinically relevant) variables is extensive. As such, data analysis and interpretations resulting from these procedures should be especially meticulous. Discussed next are three models that are commonplace in preclinical drug use research: intravenous-self administration, intracranial self-stimulation, and conditioned place preference. Tables 1 and 2 display previous studies that have assessed the behavioral effects of MDPV and 4-MMC, respectively, using these procedures. Further, below are discussions of select studies for additional exposition and detailed description to familiarize readers with the general experimental questions addressed using these procedures and the associated data path analyses.

Table 1

Reinforcing and Rewarding Effects of MDPV

Procedure	Dose	Subjects	Results	Reference
IVSA	0.05, 0.1, 0.2 mg/kg/infusion	♂Sprague-Dawley rats	All doses of MDPV maintained IVSA	Watterson et al. (2012)
	0.05 mg/kg/inf	♂Wistar rats	MDPV potency and efficacy > methamphetamine	Aarde et al. (2013a)
	0.05 mg/kg/inf	♂Wistar rats	MDPV = α-PVP as SR^+	Aarde et al. (2015a)
	0.05 mg/kg/inf	♂Wistar rats	MDPV binge rats displayed post-acquisition decrease in wheel-running	Aarde et al. (2015b)
	0.03 mg/kg/inf	♂Sprague-Dawley rats	MDPV maintained IVSA; similar to 0.5 mg/kg/inf cocaine	Schindler et al. (2016)
ICSS	0.1, 0.5, 1, 2 mg/kg	♂Sprague-Dawley rats	MDPV lowered ICSS thresholds at all doses	Watterson et al. (2012)
	0.32-3.2 mg/kg	♂Sprague-Dawley rats	MDPV was second (to methcathinone) most potent to facilitate ICSS	Bonano et al. (2014a)
	3.2 mg/kg	♂Sprague-Dawley rats	MDPV facilitated ICSS	Bonano et al. (2014b)
	0.32-10 mg/kg (+)MDPV or (-)MDPV	♂Sprague-Dawley rats	*S*(+)MDPV potency > (±)MDPV at facilitating ICSS (dose-dependent)	Kolanos et al. (2015)
CPP	0.5, 2, 5, 10, 20 mg/kg	♂C57BL/6 mice	All doses produced evidence of CPP. 5 and 10 mg/kg MDPV > amphetamine CPP at same doses	Karlsson et al. (2014)
	1, 1.8, or 3.2 mg/kg	♀ and ♂ Sprague-Dawley rats	1.8 and 3.2 mg/kg MDPV produced CPP across male and female rats	King et al. (2015a)
	1, 1.8, or 3.2 mg/kg	♂Sprague-Dawley rats	All doses produced increases in time-spent in drug-paired chamber	King et al. (2015b)

IVSA = intravenous self-administration; ICSS = intracranial self-stimulation; CPP = conditioned place preference

Table 2

Reinforcing and Rewarding Effects of 4-MMC

Procedure	Dose	Subjects	Results	Reference
IVSA	0.24 mg/kg/infusion	♂Sprague-Dawley rats	MC maintained IVSA	Hadlock et al. (2011)
	0.3 mg/kg/inf	♂Sprague-Dawley rats	MC IVSA > methamphetamine IVSA	Motbey et al. (2013)
	0.5 or 1.0 mg/kg/inf	♂Sprague-Dawley and Wistar rats	MC maintained IVSA	Aarde et al. (2013b)
	0.5 mg/kg/inf	♀Wistar rats	MC infusion rates > methylone during MDMA acquisition	Creehan et al. (2015)
	0.5 mg/kg/inf	♂Wistar rats	MC intake > MDMA or methylone during 2h access	Vandewater et al. (2015)
	0.5 mg/kg/inf	♂Wistar rats	Long-access-trained rats displayed > breakpoints during MC and methamphetamine tests	Nguyen et al. (2016)
ICSS	1, 3, or 10 mg/kg	♂C57BL/6J mice	MC potentiates brain stimulation reward	Robinson et al. (2012)
	1-10 mg/kg	♂Sprague-Dawley rats	ICSS *same as MDPV above*; MC < potent at facilitating ICSS	Bonano et al. (2014a)
	R-and *S*-isomers (1-10 mg/kg)	♂Sprague-Dawley rats	*R* produced greater ICSS facilitation than *S*	Gregg et al. (2015)
CPP	30 mg/kg	♂Sprague-Dawley rats and ♂CD-1 mice	30 mg/kg elicited CPP in rats and mice	Lisek et al. (2012)
	0.5, 2, 5, 10, or 20 mg/kg	♂C57BL/6 mice	5 and 20 mg/kg elicited CPP	Karlsson et al. (2014)
	10 or 25 mg/kg	♂Swiss CD-1 mice	10 and 25 mg/kg elicited CPP	Ciudad-Roberts et al. (2015)
	10, 100, or 500 μM	Planarians (*Dugesia dorotocephala*)	100 and 500 μM elicited CPP	Ramoz et al. (2012)
	R-isomer and *S*-isomer (5-30 mg/kg)	♂Sprague-Dawley rats	30 mg/kg *R* produced CPP; *S* did not produce CPP	Gregg et al. (2015)
	R-isomer, *S*-isomer, and racemate (10, 100, 250 μM)	Planarians (*Dugesia dorotocephala*)	10, 100, 250 μM *R* and 100 μM racemate elicited CPP	Vouga et al. (2015)
	1 or 10 μM	Brown planarians (*Dugesia Tigrina*)	1 or 10 μM failed to elicit CPP	Hutchinson et al. (2015)

MC = 4-MMC; IVSA = intravenous self-administration; ICSS = intracranial self-stimulation; CPP = conditioned place preference

***Self-administration*.** Drug self-administration procedures are useful in behavioral pharmacology research for investigating the relative reinforcing value of drugs (for review, Young & Herling, 1986). Notably, self-administration methods make available direct comparisons of the reinforcing efficacy between different drugs, or to non-drug reinforcers/activities. In addition, experimental subjects can be exposed to a variety of potential pharmacotherapeutics and environmental manipulations (i.e., non-drug manipulations) to determine if such interventions reduce response-dependent drug infusions.

Recently, extensive use of self-administration procedures has broadened understanding of the relative reinforcing value of MDPV compared to other drugs and non-drug reinforcers. For example, Watterson et al. (2012) reported the first experiment to assess the reinforcing efficacy of MDPV (0.05, 0.1, or 0.2 mg/kg/infusion) using intravenous self-administration in rats. The results revealed that all doses of MDPV supported self-administration, and that MDPV produced patterns of responding under a progressive-ratio schedule similar to methamphetamine (0.05 mg/kg/infusion). These results indicate that MDPV serves a potent reinforcer that may possess potential for drug misuse. In a similar study, Aarde, Huang, Creehan, Dickerson, and Taffe (2013) compared the relative reinforcing efficacy of 0.05 mg/kg/infusion MDPV to 0.05 mg/kg/infusion *d*-methamphetamine using intravenous self-administration procedures in male Wistar rats. The results of this study revealed that MDPV supports intravenous self-administration in male Wistar rats, and that the infusion rates and number of responses that occurred under a progressive-ratio schedule were greater with MDPV than methamphetamine.

Previous research has also investigated the reinforcing efficacy of MDPV in comparison to non-drug reinforcers. Aarde, Huang, Dickerson, and Taffe (2015) assessed in Wistar rats whether opportunities to receive 0.5 mg/kg/infusion MDPV would lessen the relative reinforcing value of access to a running wheel. The results of this study revealed that as a binge-like pattern of MDPV self-administration occurred in an individual rat, there existed a corresponding decrease in the amount of time allocated to a concurrently-available running wheel. These findings suggest that observing individual differences in acquiring MDPV self-administration is the rule rather than the exception (a fact of most self-administration experiments and of drug consumption in general), and that MDPV as a reinforcer may compete with ecologically-relevant reinforcers present in a user's environment. Further comparisons of MDPV's

reinforcing efficacy to other non-drug, but ecologically-relevant activities, seems worthwhile in light of the foregoing data.

Previous research has also investigated the relative reinforcing value of 4-MMC using self-administration procedures. In the first known report, Hadlock et al. (2011) observed that male Sprague-Dawley rats would reliably lever-press to receive 4-MMC (0.24 mg per 10μl infusion) under an FR1 schedule of drug reinforcement. In addition, rats displayed an increase in the total number of responses that produced 4-MMC delivery as the number of sessions increased. These findings demonstrate that 4-MMC supports self-administration using rodent behavioral procedures. Subsequent studies have reported similar findings as those found in Hadlock et al. (2011). For example, Motbey et al. (2013) observed that male Sprague-Dawley rats would reliably nose-poke to receive 0.03 mg/kg/infusion 4-MMC. Under a progressive-ratio schedule, the rats displayed increases in the number of active nose-pokes (i.e., nose-pokes that produced drug delivery) and infusions as the dose of 4-MMC increased. In addition, rats displayed similar values of active pokes and infusions under 4-MMC to methamphetamine, suggesting that 4-MMC may possess reinforcer efficacy similar to methamphetamine (Motbey et al., 2013).

In a recent study, Vandewater, Creehan, and Taffe (2015) compared levels of intravenous self-administration between 4-MMC, MDMA, or 3,4-methylenedioxymethcathinone (methylone) in male Wistar rats responding under an FR1 schedule of drug reinforcement. Compared to methylone- or MDMA-trained rats, 4-MMC-trained rats displayed the highest infusion levels during the acquisition phase, and the "high-preference" subgroup in the 4-MMC-trained rats (i.e., the upper portion of the median split in infusion levels among rats) also showed increases in breakpoints when responding to receive infusions of 4-MMC, MDMA, and methylone. Overall, these findings support the results of previous reports indicating that 4-MMC may possess relatively high abuse potential among users, especially in comparison with other drugs of abuse, such as MDMA and methylone (Vandewater, Creehan, & Taffe, 2015).

It is noteworthy that the aforementioned self-administration studies were conducted using male rats as experimental subjects. Comparisons of the effects of drug treatments on behavioral measures using female rats is less common in behavioral pharmacology research. Nevertheless, recent experiments have used female subjects to assess effects of synthetic cathinones on behavioral and physiological measures. Of particular interest, Creehan, Vandewater, and Taffe (2015) reported that female Wistar rats reliably

intravenously self-administered 0.5 mg/kg/infusion 4-MMC and the infusion rates were higher than 0.5 mg/kg/inf MDMA- or 0.5 mg/kg/inf methylone-trained rats. In sum, these findings support the foregoing self-administration reports that used 4-MMC as a training drug in rats, and that 4-MMC serves as a relatively potent reinforcer in a variety of self-administration preparations. Other reports that have investigated the behavioral and physiological effects of the synthetic cathinones using female subjects are discussed below.

Intracranial self-stimulation. Although the self-administration assay provides the most direct measure of evaluating the reinforcing efficacy of a drug, intracranial self-stimulation (ICSS) procedures are also common in preclinical research. Unlike the self-administration paradigm where drug delivery is response-dependent, ICSS procedures include the use of an artificial stimulus (e.g., electric current) and inferential reasoning to evaluate the rewarding value of a drug. For example, the median forebrain bundle is commonly used as the target brain region to receive electrical stimulation. This bundle is typically selected because of its well-characterized innervations with brain regions involved in drug addiction and reward, such as the nucleus accumbens. In general rodent preparations, it is typical that injections of drugs with abuse potential delivered prior to opportunities for responding to receive electrical stimulation will reduce the overall stimulation; that is, pretreatment with drugs of abuse tend to decrease subsequent response-dependent electrical stimulation. If an ICSS experiment reveals that some novel drug or drug heretofore untested reduces overall stimulation, then the drug is considered to possess abuse potential. Although the ICSS assay seems predictive of a drug's abuse potential, the findings should be interpreted with caution given that the subjects are not responding to receive the drug itself (*cf*. self-administration procedures).

In a recent study, Kolanos et al. (2015) evaluated the stereochemistry of MDPV optical isomers using ICSS procedures. The authors reported that *S*(+)-MDPV dose-dependently facilitated ICSS and was more potent that racemic MDPV under similar testing conditions. In contrast, *R*(-)-MDPV failed to affect ICSS rates at all doses tested. In addition to MDPV, previous reports have assessed the stereochemistry of 4-MMC using ICSS procedures. Gregg et al. (2015) revealed that (*R*)-4-MMC produced greater ICSS facilitation than the (*S*)-4-MMC isomer. In addition, (*R*)-4-MMC more potently released [3H]MPP+ (the radiolabeled substrate) at DAT in rat synaptosomes, compared to (*S*)-4-MMC. The authors of that study

concluded that their findings warrant further studies evaluating the neurochemistry and behavioral effects of 4-MMC optical isomers. Although noted in their discussion that some illicitly-synthesized compounds (e.g., methcathinone) contain larger portions of one enantiomer over another in mixture, there are no known published studies on the prevalence of one enantiomer of 4-MMC over another in drug mixtures, or if enantiomers are region-specific (i.e., variant drug market availability). Similar ambiguity exists for MDPV; only additional research can determine if continued investigations of synthetic cathinone stereochemistry is a pragmatic issue with these substances.

Conditioned place preference. Conditioned place preference (CPP) procedures are useful for predicting the rewarding effects of drugs produced by Pavlovian conditioning processes (although, evidence of place preference may not be unequivocally attributable to Pavlovian conditioning processes; see, Huston, de Souza Silva, Topic, & Müller, 2013). CPP procedures involve repeated intermittent pairing of drug-associated cues with a distinct environment, and subsequent assessment of a subject's time spent in that environment. As such, CPP procedures are useful for assessing whether the rewarding effects of drugs can be paired with contextual cues, and, if so, such cues may further prompt drug craving and relapse among human users. Lisek et al. (2012) observed that male Sprague-Dawley rats and male CD-1 mice injected with 30 mg/kg 4-MMC during the place conditioning trials displayed statistically greater levels of preference scores than saline-treated rats or mice, respectively. In a later study, Karlsson, Andersson, Kronstrand, and Kugelberg (2014) investigated whether 4-MMC, MDPV, and methylone would produce CPP in male C57BL/6 mice. Results from this study revealed that mice injected with 0.5 or 20 mg/kg 4-MMC, or any dose of MDPV (0.5, 2, 5, 10, or 20 mg/kg) during the place conditioning trials displayed statistically greater levels of drug-paired preference scores compared to saline-treated mice. In addition, mice injected with 5 or 10 mg/kg MDPV displayed greater levels of preference scores compared to mice injected with similar doses of amphetamine.

Similar to the findings reported by Karlsson, Andersson, Kronstrand, and Kugelberg (2014), King, Wetzell, Rice, and Riley (2015) observed in male Sprague-Dawley rats that 1.0, 1.8, and 3.2 mg/kg MDPV produced statistical differences in the percent of time in the drug-paired side across conditioning sessions (i.e., preconditioning and post-conditioning); however, the authors noted that the rats did not actually spend more time in the drug-paired side than the unpaired side during the post-conditioning session. Moreover,

the effects were not dose-dependent. As such, this experiment also demonstrates that results should be interpreted with caution because evidence of statistical effects may be due to data handling rather than clear drug effects.

Thus far, the foregoing studies on the synthetic cathinones have included rodents as experimental subjects. Nevertheless, recent interests have shifted to consider organisms with relatively 'simpler' nervous systems (i.e., fewer neurons, well-characterized genomes). Recently, Hutchinson, Prados, and Davidson (2015) assessed in brown planaria (*Dugesia Tigrina*) whether 1 or 10 μM of cocaine or 4-MMC would elicit CPP. The results revealed that both doses of cocaine produced evidence of place preference, however neither dose of 4-MMC elicited place preference in the subjects. Contrary to the results of Hutchinson, Prados, and Davidson (2015), Ramoz et al. (2012) observed that 100 and 500 μM of 4-MMC produced evidence of place preference in planaria (*Dugesia dorotocephala*). Discrepancies between the results of this study and Hutchinson, Prados, and Davidson (2015) are likely due to the different doses of 4-MMC tested, the different planaria strain used, and the procedures used for drug conditioning (e.g., Ramoz et al., 2012) evaluated CPP among the subjects in a single day and Hutchinson et al. used multiple days of drug conditioning). Nonetheless, these findings together suggest that the effects of 4-MMC on place preference in planaria are dose-dependent and, importantly, that similar responses to 4-MMC may occur across species. It is admirable that these studies demonstrated comparable CPP effects in invertebrates, such as planaria, to experimental subjects that are generally "by default" selected as experimental subjects (viz. rodents). Toward the bioethical goal of reduction, refinement, and replacement among non-human subjects used in scientific research, a shift from using vertebrates to invertebrates in drug use research is a commendable endeavor.

Drug Discrimination

Background. Findings from state-dependent learning experiments demonstrated that experimental subjects could display one response in the presence of a drug, and another response in its absence. Specifically, early findings including state-dependent learning procedures revealed that a drug's interoceptive stimulus effects affected the acquisition and performance of learned responses—subjects would display better performance if the stimulus conditions (e.g., drug exposure) during the acquisition

phase were identical to the stimulus conditions in the performance phase[3]. From these previous state-dependent learning studies, emerged drug discrimination (DD) methods that would eventually develop into a validated assay for measuring a drug's discriminative stimulus effects (see Colpaert, 2011).

Over decades, the DD paradigm has undergone considerable procedural transformations (see Glennon & Young, 2011b). Early DD preparations consisted of a T-maze apparatus (e.g., Overton, 1982) which required subjects (typically rodents) to discriminate between the "left" or "right" arm of the maze conditional upon the presence or absence of a drug's interoceptive effects during the testing session. Single-lever operant procedures using operant conditioning chambers soon replaced the T-maze preparation to improve and standardize DD procedures in general, and to provide additional measurement of a drug's behavioral effects (e.g., a drug's effects on rate of responding). Operant conditioning chambers of variable dimensions and components were also developed to test behavioral effects of drugs in several species, such as rats, mice, rabbits, pigeons, and primates. Accordingly, the foregoing T-maze procedures seemed to have been abandoned by most drug discrimination researchers, although, rarely, such procedures are used in current times.

Experimental research using DD procedures increased considerably in the mid-20th century. Researchers across a range of disciplines (e.g., behavioral pharmacologists, medicinal chemists) further expanded the DD assay to accommodate several training drugs (e.g., equipping an operant conditioning chamber with multiple levers), test whether a drug's behavioral effects are conditional on variable environmental factors (e.g, schedules of reinforcement), and test other behavioral processes (e.g., respondent relations and conditioned taste aversion procedures). As mentioned, a common DD preparation comprises two-lever rodent operant conditioning chambers using food, water, or sweetened milk as reinforcers for lever-press responding. The most common schedules of reinforcement used in DD experiments are fixed-ratio schedules, although experimenters have tested using a variety of other schedules and schedule combinations (second-order schedules) (see Glennon & Young, 2011b).

The most common uses of DD methods are to characterize drugs' interoceptive effects or reveal receptor isoforms that are involved in mediating their discriminative stimulus properties. Drug

[3] In the behavioral literature, a decrease in performance as a function of dissimilar stimulus conditions during acquisition and performance can be referred to as "stimulus-change decrement".

classification is valuable for determining the interoceptive effects of a novel or emerging drug. For example, if a novel β-ketoamphetamine appears in the public domain, it is valuable to know if the drug produces subjective effects in users similar to other illicit drugs, such as methamphetamine, cocaine, or MDMA. Most DD experiments include stimulus substitution tests (or stimulus generalization tests) to determine if the interoceptive cue produced by some other drug of interest resembles the cue produced by the training drug. Specifically, in the case of a rodent operant chamber using mechanical levers, stimulus substitution tests assess the extent to which a test drug exerts stimulus control over lever-press responding in comparison to a training drug's control over lever-press responding. Moreover, DD procedures are valuable for assessing whether a specific dose of a novel training drug tested using specified procedures produces effects similar to other well-characterized drugs of abuse. Such information could be useful for informing the public, private, and government sectors about the drug's effects. In addition, drugs with effects known to be centrally-mediated (i.e., effects produced by stimulation in the central nervous system) can be distinguished from drugs with peripherally-mediated effects using DD procedures.

As mentioned, a second use of DD procedures is to reveal receptor isoforms that are involved in mediating a drug's discriminative stimulus effects. For example, if test doses of cocaine produce full substitution to a training dose of the aforementioned β-ketoamphetamine, then it is possible that the stimulus effects produced by the β-ketoamphetamine may be mediated by dopamine receptor activation (note: drugs with variable physiological effects can produce comparable interoceptive effects). Moreover, this possibility can be further assessed by pretreating the subjects trained on the dose of the β-ketoamphetamine with drugs that possess selective binding affinities to dopamine receptor isoforms. For example, experimental subjects can be injected with doses of a selective D_2 receptor antagonist (e.g., haloperidol) prior to receiving doses of the training drug. As long as the experimenter properly accounts for the pharmacokinetics of haloperidol and the β-ketoamphetamine, then the subject's responses may reflect whether D_2 receptor activation is involved in mediating the training drug's stimulus cue (e.g., if there is a downward [reduction in efficacy or maximal effect] or leftward shift [reduction in potency] in the training drug's dose-response curve). Overall, stimulus blockade studies may assist in future research efforts devoted toward developing pharmacotherapies for drug users and inform researchers of potential protein receptors involved in mediating a drug's effects.

On a final note, it is noteworthy that rodents and pigeons will respond to a drug's interoceptive effects during DD procedures in a manner similar to other species, including humans (e.g., in experimental settings where humans are pushing buttons to receive points or money under some specified schedule of reinforcement). Therefore, DD methods are useful for investigating a drug's effects when such methods are precluded for use in humans, which is true in most general cases. It is also worth emphasizing that a variety of environmental variables are known to modulate a drug's effects (e.g., Poling, 2000), suggesting that a drug's interoceptive "cue" is differentially expressed depending upon the environmental context in which drug exposure takes place. Nevertheless, relatively few experimental procedures, beyond DD, exist that permit investigations of a drug's possible interoceptive effects. For more comprehensive historical and procedural descriptions of the DD paradigms, readers are referred elsewhere (Solinas, Panlilio, Justinova, Yasar, & Goldberg, 2006; Young, 2009; Glennon & Young, 2011b).

Quantal versus graded dose-response effects. Drug discrimination data are generally analyzed (and conceptualized) using one of two approaches. The first, referred to as the "quantal" approach, states that subjects' responses occur in an all-or-none fashion (a nominal dependent variable). That is, the discriminative cue is either present or it is not (compare a drug's interoceptive effects to an on and off switch). In contrast, the second, referred to as the "graded" approach, states that subjects' responses are proportional to a dose of a drug (an ordinal or interval dependent variable). That is, subjects will display greater levels of stimulus generalization as the dose of a drug increases (assuming the test drug produces an interoceptive cue that resembles the cue produced by the training drug). The graded approach seems most common in the recent drug discrimination literature, possibly due to the development of simplified statistical procedures that can easily analyze graded dose-response functions (for a brief review on this subject, see Glennon & Young, 2011c). Nevertheless, theoretical debates between individuals who promote these approaches has prompted interesting discussions and conceptualizations about drug discrimination data (see the Discussion below for more information regarding quantal versus graded drug discrimination data).

MDPV and 4-MMC in drug discrimination. Relatively few studies have investigated the discriminative stimulus effects of MDPV and 4-MMC using DD methodology. In the first known study to use a dose of MDPV as a training drug, Fantegrossi, Gannon, Zimmerman, and Rice (2013) observed in 0.3

mg/kg MDPV-trained mice responding under a FR 20 – 10-s time-out procedure of sweetened milk reinforcement that racemic MDPV, racemic MDMA, and methamphetamine produced full substitution. Contrariwise, a cannabinoid receptor CB_1 and CB_2 agonist, JWH-018, and an opioid receptor agonist, morphine, failed to produce full substitution. These authors used a cumulative dosing procedure to generate a complete dose-response curve for each test drug in a single testing session. Each test drug dose-dependently reduced response rate as well. Overall, these authors concluded that the interoceptive effects of MDPV may be similar to the cues produced by methamphetamine and MDMA.

Similar to the study above, Varner et al. (2013) revealed that in 3.2 mg/kg 4-MMC-trained Long-Evans rats, MDMA produced full substitution, and cocaine and methamphetamine produced high partial substitution (75.86% and 72.86% drug-appropriate responding, respectively). Contrariwise, morphine, fenfluramine (serotonin releaser), and phencyclidine (glutamate NDMA receptor antagonist) failed to produce complete generalization in the 4-MMC-trained rats. In addition, Varner et al. pretreated rats with a selective dopamine D_2 receptor antagonist (haloperidol) to evaluate if the D_2 receptor was involved in mediating 3.2 mg/kg 4-MMC's discriminative cue. The results of the blockade tests revealed that 0.032 and 0.1 mg/kg haloperidol failed to block the 3.2 mg/kg 4-MMC cue. Thus, Varner et al. suggested that dopamine D_2 receptor activation may not be involved in mediating 4-MMC's subjective effects.

Other studies have evaluated whether MDPV or 4-MMC would substitute in rodents trained under other drugs or drug mixtures. Gatch, Taylor, and Forster (2013) found that in 10 mg/kg cocaine- or 1 mg/kg methamphetamine-trained mice that MDPV and 4-MMC produced full substitution. Given these findings, Gatch et al. concluded that MDPV and 4-MMC produce behavioral effects (i.e., interoceptive effects) that are comparable to cocaine and methamphetamine. In a later study, Harvey and Baker (2016) demonstrated that MDPV produced full substitution in male Sprague-Dawley rats trained to discriminate a mixture of MDMA (1.5 mg/kg) and *d*-amphetamine (0.5 mg/kg) from saline, but not in another group of rats trained to discriminate 1.5 mg/kg MDMA from saline. In addition, 4-MMC produced full substitution in both of the foregoing training groups. Overall, the authors of that study suggested that, unlike MDPV, the interoceptive effects of 4-MMC might involve serotonin release. Further research using compounds with selective affinities for serotonin receptors may support this hypothesis.

Current Research Objective

To further characterize the discriminative stimulus effects of MDPV and 4-MMC, groups of male Sprague-Dawley rats were trained to discriminate 0.3 mg/kg MDPV or 1.0 mg/kg 4-MMC from saline, in a two-lever drug discrimination task. The main objective of this experiment was to generate dose-response curves of the following test drugs in these training groups: MDPV, 4-MMC, *d*-amphetamine, (+)-methamphetamine, (-)-cocaine, MDMA, (+)-LSD, and (+)-fenfluramine.

METHODS

Subjects

Sixteen male Sprague-Dawley rats weighing 350-400g (Charles River Laboratories Inc., Kingston, NY, USA) were singly housed in polycarbonate cages with corncob bedding (ENVIGO, Madison, WI, USA) in a temperature and humidity controlled vivarium maintained on a 12/12h light/dark cycle (lights on at 0800h). Rats were maintained at 85-90% of free-feeding weights through restricted daily food rations (LabDiet®, PMI® Nutrition LLC., Brentwood, MO, USA), but access to water was unrestricted in the home cages. All procedures were conducted 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 at Western Michigan University.

Apparatus

Eight computer-operated standard three-lever rat operant chambers (ENV-100; MED Associates, St. Albans, VT, USA) contained in sound-attenuating cabinets equipped with fans for ventilation and masking noise were used for all training and testing sessions. The operant chambers consisted of an acrylic top, side, and door panel, with other walls and components made of stainless steel. A houselight (28V) located near the top of the rear stainless panel of the chamber provided illumination during all sessions. Three retractable levers were located above a stainless steel barred floor. Two levers were equidistant from a center food magazine on the front wall, while a third lever was located directly beneath the food magazine. Food reinforcers consisted of 45mg powderless food pellets (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), (±)-mephedrone HCl, (+)-methamphetamine HCl, (-)-cocaine HCl, (+)-lysergic acid diethylamide tartrate, and (+)-fenfluramine HCl were provided by the National Institute on Drug Abuse Drug Control Supply Program (Bethesda, MD, USA). *d*-Amphetamine hemisulfate was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Each drug solution was prepared by dissolving salt in 0.9% (wt/vol) bacteriostatic saline. All doses are expressed as weight of salt. Drug injections were performed via intraperitoneal (ip) injections with a 15-min pre-session injection interval in a 1ml/kg injection volume.

Operant Training Procedures

All chambers and levers were wiped clean with 35% isopropyl alcohol after every experimental session to attenuate olfactory cues remaining on the levers and in the testing environment (Extance & Goudie, 1981). Additionally, on all training and testing days, rats received their daily food rations at variable intervals post-session (generally 5-10-min post-session) to attenuate post-session suppression of responding (Smethells, Fox, Andrews, & Reilly, 2012).

Magazine training. All subjects were placed in the operant chambers for a single, 60-min session where food was delivered under a variable-time 60-s schedule of reinforcement. The criterion for proceeding to lever press training consisted of each rat consuming all pellets from the magazine by the end of the 60-min session. All subjects proceeded to lever press training after one session.

Lever press training. After the initial magazine training session, lever press training sessions began. During lever press training, each rat was placed in an operant chamber with only the center lever extended. Food deliveries were response-dependent under a fixed-ratio (FR) schedule of reinforcement. All subjects were required to produce five food pellets under an FR schedule before the FR requirement increased by one (e.g., five total responses under FR1, followed by five responses under FR2). Lever press training sessions consisted of five training phases: 1) FR 1-FR 5, 2) FR 5-FR 10, 3) FR 10-FR 15, 4) FR 15-FR 20, and 5) FR 20. Note that each session began with the maximum FR schedule obtained in the previous session. These sessions were selected to engender reliable lever presses under the terminal FR 20 schedule. In the event a subject did not display reliable responding under a particular FR schedule, the FR schedule was incrementally reduced until responding occurred again. Each session lasted 30-min or until a

subject produced 60 food pellets, whichever occurred first. All subjects proceeded to errorless training after 7-10 lever-press training sessions.

Errorless (single lever) training. During single lever training sessions, rats were injected (ip) with either drug (D) or vehicle (V) and placed in the chamber with only the condition-appropriate lever extended. Drug injections consisted of 0.3 mg/kg MDPV (N = 8) or 1.0 mg/kg 4-MMC (N = 8) specific to the training group, and vehicle injections consisted of 0.9% bacteriostatic saline for both groups. The assignment of each drug condition on the two levers (i.e., either the left lever or the right lever) was counterbalanced across half of the subjects within each training drug condition. All rats were exposed to six errorless training sessions in the following order: V,V,D,D,V,D. Responses were reinforced under an FR 20 schedule. In the event a subject did not reliably respond (i.e., produced few food pellets relative to the other subjects within the same training group), the FR requirement was incrementally reduced until reliable responding once again occurred under the FR 20 schedule. All rats were responding on an FR 20 schedule by the last training session (sixth training session) within each drug condition.

Discrimination training. During discrimination training sessions, rats were injected with either D or V and placed in the chamber with both levers extended. Drug and vehicle training sessions were implemented in a pseudorandom order, under the limitation that no animal received more than two consecutive drug or two consecutive vehicle sessions throughout the study. For example, in any six-day period, D and V sessions occurred in one of the following orders: VVDDVD, DDVVDV, VDDVDD, DDVDDV, DVVDDV, or DVDVVD. Consistent with errorless training, lever assignments (either D or V) remained constant for each rat, and counterbalanced across half of the rats within each training group. The criteria for discrimination training required rats to emit ≥80% condition-appropriate lever responses prior to the delivery of the first reinforcer and for the remainder of the training session for at least 8 of 10 consecutive discrimination training sessions.

Stimulus substitution testing. Stimulus substitution tests commenced when each subject met the criteria for discrimination training above. A test session occurred after a subject completed no less than one drug training session and one vehicle training session, where in each session it emitted ≥ 80% condition-appropriate responding prior to the first FR and throughout the duration of the session. If a subject did not meet these criteria, training sessions continued until it met these criteria under each stimulus condition for

two consecutive sessions. Doses of substitution test compounds were counterbalanced across subjects in each training group. Substitution tests occurred no more than two times per week with the compounds presented Table 3:

Table 3

Compounds for Substitution Tests

Test Compound	Dose (mg/kg)
MDPV HCl	0, 0.01, 0.03, 0.1, 0.3, 1, 3
4-MMC HCl	0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10
d-Amphetamine hemisulfate	0, 0.03, 0.1, 0.3, 1, 3
(+)-Methamphetamine HCl	0.03, 0.1, 0.3, 1, 3
(-)-Cocaine HCl	0, 0.1, 0.3, 1, 3, 10
MDMA HCl	0, 0.03, 0.1, 0.3, 1, 3
(+)-LSD tartrate	0.01, 0.03, 0.1, 0.2
(+)-Fenfluramine HCl	0.03, 0.1, 0.3, 1, 3

Drugs were tested in the order depicted above.

During stimulus substitution testing, rats were injected with a dose of one of the following compounds, returned to their home cages, and placed into the experimental chamber after the 15-min pre-session injection interval had elapsed. A test session ended after a subject completed 20 consecutive responses on either lever or 20-min elapsed, whichever occurred first. All test sessions were conducted under extinction and were treated as a D session (or V session for saline tests). The doses for all substitution test compounds were taken from previous drug discrimination studies conducted in-house, which tested the drug in the same species, via the same route of administration (ip).

Data Analysis

Acquisition of drug stimulus control was determined by the number of discrimination training sessions required to reach criterion among each training group (see above). Learning curves for each training group were created to display mean percent drug-lever selection during the first FR as a function of training sessions. Percent drug-lever selection was determined by dividing the number of responses on the D lever by the total number of responses emitted up to completion of the first FR 20 on either lever. Nonparametric Kaplan-Meier survival curves were generated to display the number of training sessions among the two groups. Differences in survival curves were analyzed using a Log-Rank (Mantel-Cox) test. In addition, an intercorrelation matrix was created to assess the strength of relationships among the

following set of variables within each training group: injection (1=drug, 0=vehicle), percent drug-lever selection during the first FR, rate of responding during the first FR, latency to complete the first FR, and the number of reinforcers (SR+) earned throughout the 20 min session. For the foregoing intercorrelation matrix, only data recorded during the first 20 training sessions within each training group were used for analysis.

The lever (i.e., training drug or saline vehicle) on which the first FR 20 was completed, percent drug-lever responding and responses per second were obtained for each test session. Percent drug-lever selection and responses per second were reported as means (± *SE*) in dose-response curves. Full substitution was considered 80% or greater drug-lever selection, partial substitution was considered greater than 20% and less than 80% drug-lever selection, and less than 20% drug-lever selection was considered no substitution. The potencies of MDPV, 4-MMC, *d*-amphetamine, (+)-methamphetamine, (-)-cocaine, MDMA, (+)-LSD, or (+)-fenfluramine were determined by effective-dose (ED_{50}) values. ED_{50} values (with a range set 0-100%) were calculated by fitting linear functions to the percent drug-lever selection measures for each compound using a least squares regression analysis. Linear portions of dose-response curves, including not more than a single dose producing <20% drug-lever selection and not more than a single dose producing >80% drug-lever selection, were used to compute regression equations. Predicted ED_{50} values were then computed for each test dose-response curve using the calculated regression equations and 95% confidence intervals were attached to each predicted value. Further, ED_{50} values were only computed if, in addition to a substitution test compound producing full substitution, at least six of the eight subjects within each training group completed the FR 20 on either lever during a test session. For all dose-response curves, the percent substitution value for the vehicle (V) test was determined by calculating the average of all V substitution tests obtained in the experiment for each training group (see Table 3). In addition to the foregoing data analysis that treated the data as graded, data were analyzed as quantal by computing the percentage of subjects that selected the D lever (i.e., displayed >80% drug-appropriate responding) for each dose of a test compound (also note that subjects were required to complete FR 20 on either lever to be included in quantal dose-response calculations). As such, graded and quantal dose-response curves were plotted into a single graph for visual analysis. Finally, the maximum mean percent substitution values for each test drug obtained from the 0.3 mg/kg MDPV training group were correlated to the maximum mean

values for each test drug obtained from the 1.0 mg/kg 4-MMC training group using a Pearson *r* correlation analysis.

Response rates were presented as the number of responses emitted per second divided by the total session time. Response rate data associated with each stimulus substitution test drug were analyzed by one-way repeated-measures analysis of variance (RMANOVA) followed by Dunnett's multiple comparisons tests that compared each dose of a test drug to V. Similar to the percent substitution value for V, the response rate value for V was determined by calculating the average of all response rate values obtained during V substitution tests in the experiment for each training group (see Table 3). Statistical significance was identified at $p \leq .05$. Computations of ED_{50} values, Pearson *r* correlation, and response rate data were performed using PRISM GraphPad Version 7 software (La Jolla, CA, USA), and intercorrelation matrices were constructed using IBM SPSS Version 22 (Armonk, NY, USA). All graphical displays were created using PRISM GraphPad Version 7 software (La Jolla, CA, USA).

RESULTS

Acquisition of Drug Stimulus Control

A major objective of the present study was to determine if rats could reliably discriminate 0.3 mg/kg MDPV or 1.0 mg/kg 4-MMC from saline. Figure 1 and Figure 2 display the learning curves of two separate groups of eight rats trained to discriminate 0.3 mg/kg MDPV or 1.0 mg/kg 4-MMC from vehicle, respectively. Each data point represents the mean (±*SE*) for each percent drug-lever selection prior to the delivery of the first reinforcer in rats responding under an FR 20 schedule of food reinforcement. Note in Figure 1 and Figure 2 that both drug and saline sessions are plotted as percent drug-lever selection. Two subjects in the 4-MMC training group (Red 2 and Red 5) returned to discrimination training after displaying poor stimulus generalization in several stimulus substitution tests. As evident in Figures 1 and 2, as the number of the training sessions continued, the separation between the percent-drug substitution values (i.e., drug responses appearing above the 80% dashed line) and non-drug responses approaching or falling below the 20% dashed line become more evident, reflecting the development of drug stimulus control.

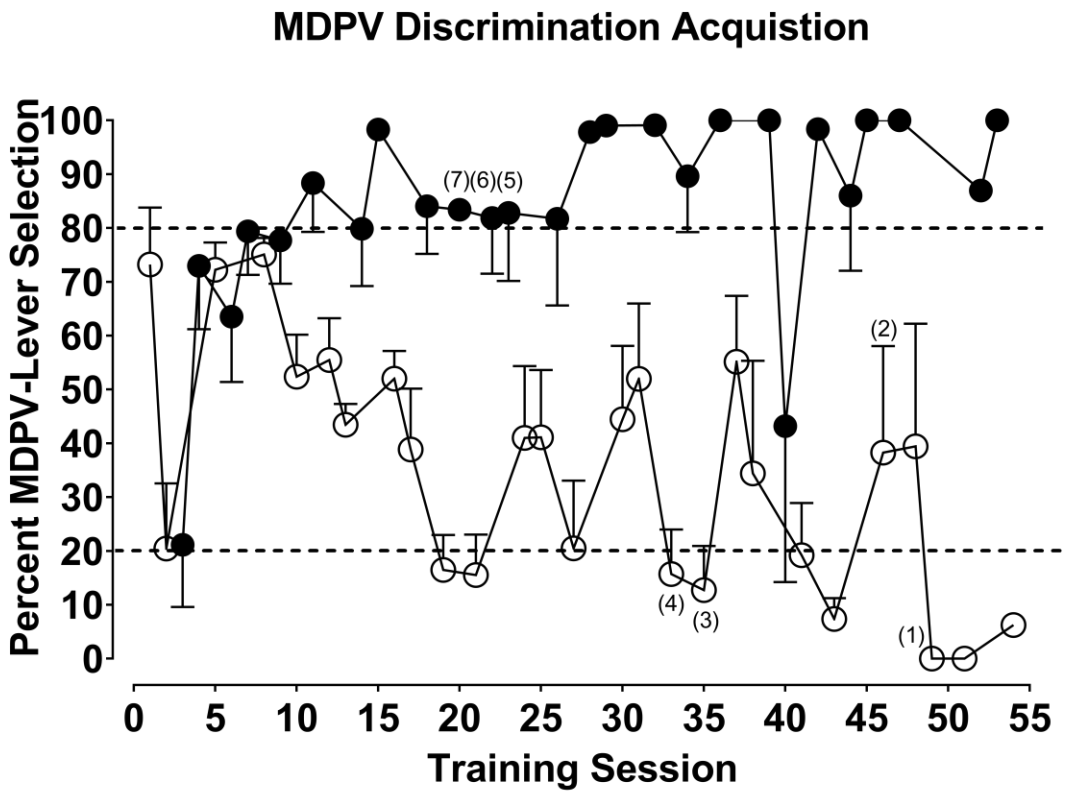

Figure 1. Learning curve of rats trained to discriminate 0.3 mg/kg MDPV from saline vehicle. Filled circles (●) represent the effect of 0.3 mg/kg MDPV and open circles (○) represent response of saline vehicle (means ± *SE*). After session 20, sample size is less than 8 on some training sessions due to some subjects having met the discrimination criteria (the number of rats is indicated in parenthesis, otherwise the number of rats was equal to 8).

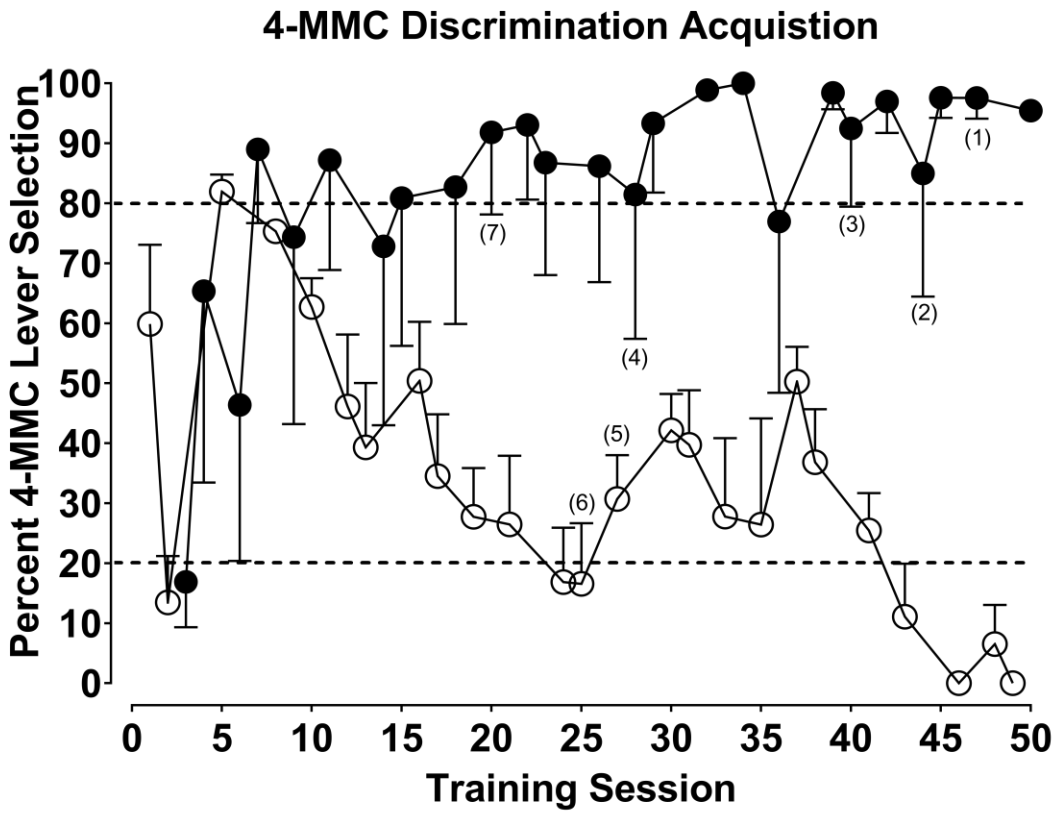

Figure 2. Learning curve of rats trained to discriminate 1.0 mg/kg 4-MMC from saline vehicle. Filled circles (●) represent the effect of 1.0 mg/kg 4-MMC and open circles (○) represent response of saline vehicle (means ± *SE*). After session 20, sample size is less than 8 on some training sessions due to some subjects having met the discrimination criteria (the number of rats is indicated in parenthesis, otherwise the number of rats was equal to 8).

Figure 3 displays a survival plot depicting when each rat reached acquisition training criteria within each training group. The 0.3 mg/kg MDPV training group met the training criteria in an average of 35.38 sessions (*SD* = 13.5), and the 1.0 mg/kg 4-MMC training group met the training criteria in an average of 37.40 sessions (*SD* = 16). As mentioned above, two subjects in the 4-MMC training group returned to discrimination training following a few substitution tests wherein poor drug stimulus control over responding was evident (i.e., inconsistent percent drug-lever selection across training sessions and substitution test sessions). As such, the survival plot below, but not the foregoing 4-MMC group learning curve, includes the additional discrimination training sessions required for one of these two subjects. A Log-Rank (Mantel-Cox) test performed on the survival curves in Figure 3 revealed no difference in the number of training sessions required to reach criteria between the MDPV and 4-MMC training drug groups, [$\chi^2(1) = 0.05$, $p > .05$].

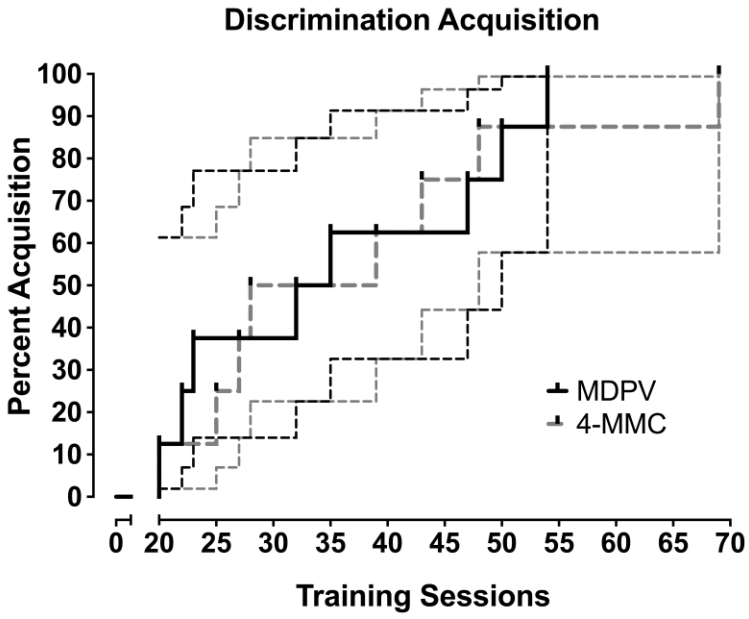

Figure 3. Nonparametric Kaplan-Meier survival curves (± 95% confidence interval [CI]) displaying the number of training sessions required for each training group to reach training criteria (note the scale break along the abscissa). The smaller, dotted black lines above and below the survival curves represent the CI for the MDPV training group; the smaller, dotted grey lines above and below the survival curves represent the CI for the 4-MMC training group. A vertical increase capped with a solid rectangle indicates a subject reaching criterion in the associated training group.

Supplementary Training Dependent Measures

Tables 4 and 5 present the intercorrelation matrices for the 0.3 mg/kg MDPV and 1.0 mg/kg 4-MMC training groups, respectively. In the 0.3 mg/kg MDPV training group, only data collected from 19 of the 20 training sessions were used in constructing the intercorrelation matrix because on one occasion two subjects failed to complete an FR during the entire 20 min training session. As such, the variables recorded during that session were omitted from the analysis to reduce the effects of outliers on the strength of the correlations (i.e., the effects of the deviant *latency to complete first FR* values). Nevertheless, in the 0.3 mg/kg MPDV-trained rats, statistically significant positive correlations were observed among the injection and percent, and SR+ and percent variables; significant negative correlations were observed among the injection and rate, latency and percent, and SR+ and latency variables. In the 1.0 mg/kg 4-MMC-trained rats, statistically significant positive correlations were observed among the SR+ and percent, and SR+ and rate variables; significant negative correlations were observed among the SR+ and latency variables.

Table 4

Intercorrelation Matrix of Variables in 0.3 mg/kg MDPV Training Group

	Injection	**Percent (1st FR)**	**Rate (1st FR)**	**Latency (1st FR)**	**SR+**
Injection	1				
Percent (1st FR)	**.703****	1			
Rate (1st FR)	**-.615****	-.373	1		
Latency (1st FR)	-.248	**-.703****	-.125	1	
SR+	.311	**.640****	.143	**-.821****	1

** $p < .01_{2\text{-tail}}$

Table 5

Intercorrelation Matrix of Variables in 1.0 mg/kg 4-MMC Training Group

	Injection	**Percent (1st FR)**	**Rate (1st FR)**	**Latency (1st FR)**	**SR+**
Injection	1				
Percent (1st FR)	.431	1			
Rate (1st FR)	-.006	.255	1		
Latency (1st FR)	.061	**-.729****	-.368	1	
SR+	.202	**.695****	**.525***	**-.798****	1

*$p < .05_{2\text{-tail}}$ **$p < .01_{2\text{-tail}}$

Substitution Tests

Individual dose-response curves generated from substitution tests are grouped together in Figures 4 through 7 based on similar pharmacological effects. However, it should be noted that the presentation order of these figures does not coincide with the order in which the subjects received the test drug compounds. The substitution test order is indicated in Table 3 within the Methods.

0.3 mg/kg MDPV training group

MDPV. The percent MDPV-lever selection and response rates under MDPV (0.01 – 0.3 mg/kg) are displayed in Figure 4A. MDPV dose-dependently increased MDPV-lever selection up to the 0.3 mg/kg training dose, which was the only dose to produce full substitution (100% ± 0 *SE*) (ED_{50} = 0.06 mg/kg; 95% Confidence Interval [CI] = 0.03 – 0.11 mg/kg). A RMANOVA revealed a statistically significant effect of MDPV on responses per second [$F(4, 28) = 3.06$, $p = .03$, $\eta^2 = 0.18$]. Dunnett's multiple comparisons tests failed to detect where the statistical differences occurred among the response rate values.

d-Amphetamine. The percent MDPV-lever selection and response rates under *d*-amphetamine (0.03 – 1.0 mg/kg) are displayed in Figure 4B. *d*-Amphetamine dose-dependently increased MDPV-lever selection up to the 1.0 mg/kg dose. Both the 0.3 (80.46% ± 12.84) and 1.0 (100% ± 0) doses of *d*-amphetamine fully substituted for 0.3 mg/kg MDPV (ED_{50} = 0.14 mg/kg; 95% CI = 0.08 – 0.24 mg/kg). A RMANOVA failed to reveal a statistically significant effect of *d*-amphetamine on responses per second [$F(4, 28) = 2.34$, $p = .08$].

(+)-Methamphetamine. The percent MDPV-lever selection and response rates under (+)-methamphetamine (0.03 – 1.0 mg/kg) are displayed in Figure 4C. (+)-Methamphetamine dose-dependently increased MDPV-lever selection up to the 1.0 mg/kg (+)-methamphetamine dose. Both the 0.3 (86.87% ± 11.22) and 1.0 (97.86% ± 2.14) doses of methamphetamine fully substituted for 0.3 mg/kg MDPV (ED_{50} = 0.12 mg/kg; 95% CI = 0.07 – 0.22 mg/kg). A RMANOVA failed to reveal a statistically significant effect of methamphetamine on responses per second [$F(4, 28) = 2.59$, $p = .058$].

(-)-Cocaine. The percent MDPV-lever selection and response rates under (-)-cocaine (0.1 - 10 mg/kg) are displayed in Figure 4D. Cocaine dose-dependently increased MDPV-lever selection up to the 10 mg/kg cocaine dose, which was the only dose to fully substitute for 0.3 mg/kg MDPV (ED_{50} = 0.73 mg/kg;

95% CI = 0.37 – 1.47 mg/kg). A RMANOVA failed to reveal a statistically significant effect of cocaine on responses per second [$F(5, 35) = 1.129$, $p = .36$].

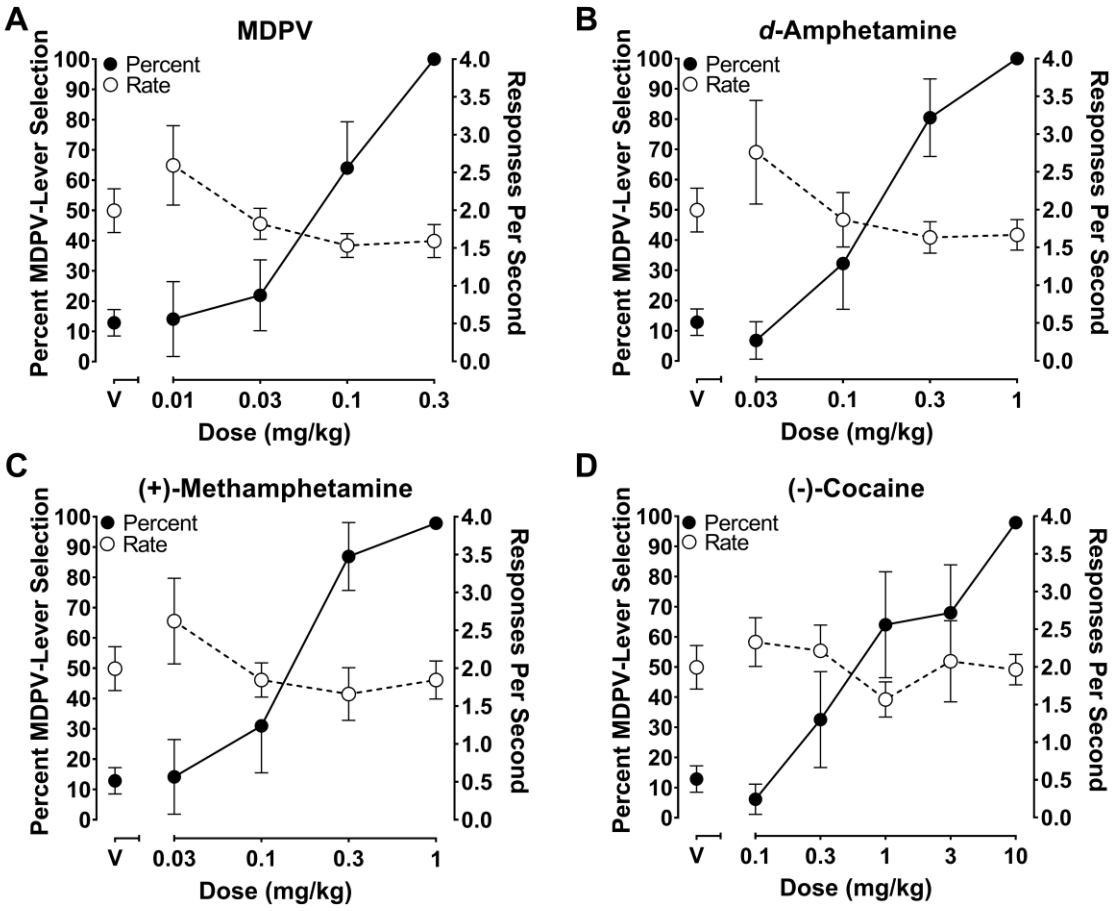

Figure 4. Results of substitution tests with doses of (A) MDPV, (B) *d*-amphetamine, (C) (+)-methamphetamine, and (D) (-)-cocaine in rats trained to discriminate 0.3 mg/kg MDPV from saline. Mean (±*SE*) percent MDPV-lever selection (left ordinate) and mean (±*SE*) responses per second (right ordinate).

Figure 5 presents a comparison between the foregoing graded dose-response curves for MDPV, *d*-amphetamine, (+)-methamphetamine, and (-)-cocaine to the same dose-response curves analyzed under a quantal approach, in rats trained to discriminate 0.3 mg/kg MDPV from saline. Note the similarity of the dose-response curves obtained between the percent MDPV-lever selection metric (graded) and the percent subjects selecting MDPV lever metric (quantal).

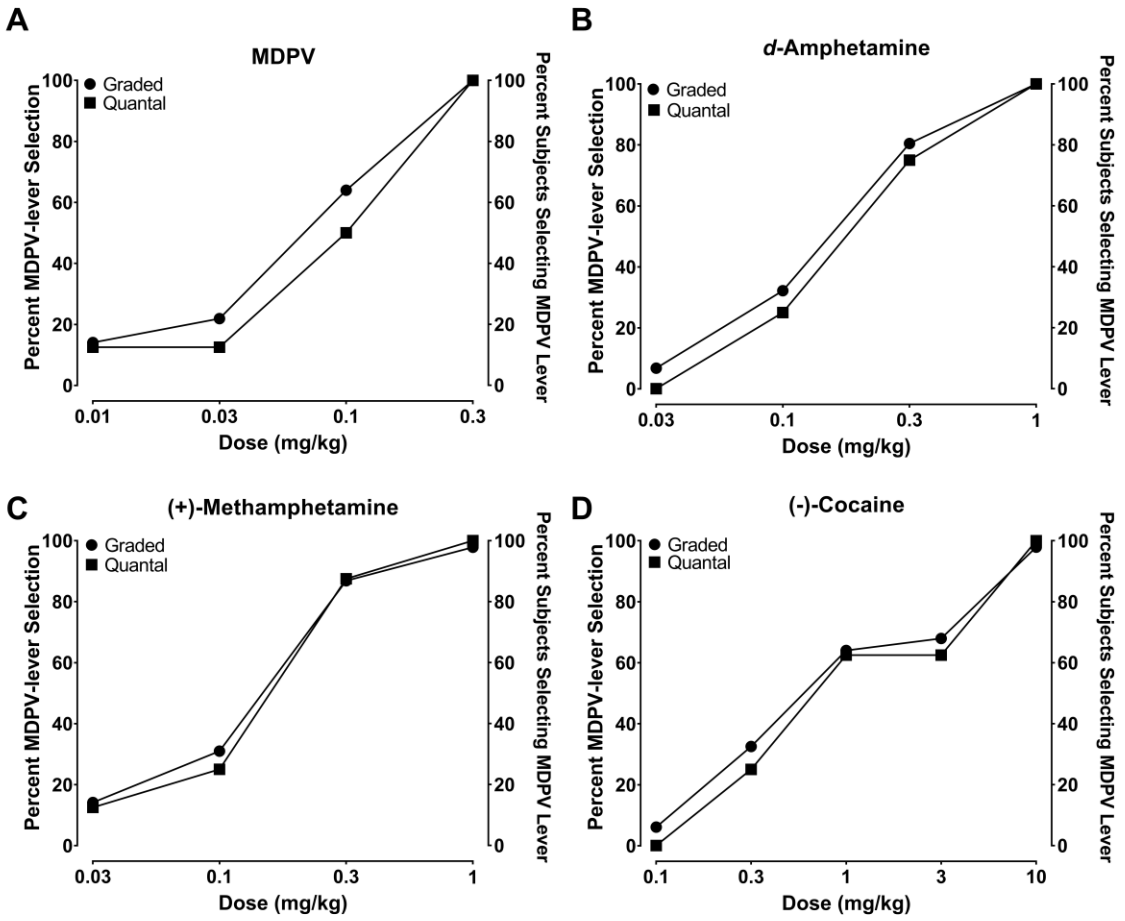

Figure 5. Quantal and graded results of substitution tests with doses of (A) MDPV, (B) *d*-amphetamine, (C) (+)-methamphetamine, and (D) (-)-cocaine in rats trained to discriminate 0.3 mg/kg MDPV from saline. Mean percent MDPV-lever selection (●, graded, left ordinate) and percent subjects selecting MDPV lever (■, quantal, right ordinate).

4-MMC. The percent MDPV-lever selection and response rates under 4-MMC (0.03 - 10.0 mg/kg) are displayed in Figure 6A. 4-MMC dose-dependently increased MDPV-lever selection with the 10 mg/kg dose (n = 6) producing partial substitution (77.26% ± 12.46). Higher doses of 4-MMC were not tested. A RMANOVA revealed a statistically significant main effect of 4-MMC on responses per second [$F(6, 42) = 15.22$, $p < .0001$, $\eta^2 = 0.49$]. Dunnett's multiple comparisons tests revealed that the 3 and 10 mg/kg doses of 4-MMC produced decreases in responses per second compared to V.

MDMA. The percent MDPV-lever selection and response rates under MDMA (0.1 – 3.0 mg/kg) are displayed in Figure 6B. MDMA did not produce notable dose-dependent increases on MDPV-lever selection, and the highest dose tested, 3 mg/kg MDMA (n = 7), only produced low partial substitution (26.62% ± 14.52). A RMANOVA revealed a statistically significant effect of MDMA on responses per second [$F(4, 28) = 5.57$, $p = .002$, $\eta^2 = 0.25$]. Dunnett's multiple comparisons tests revealed that the 3 mg/kg dose of MDMA produced decreases in responses per second compared to V.

(+)-LSD. The percent MDPV-lever selection and response rates under (+)-LSD (0.01 – 0.1 mg/kg) are displayed in Figure 6C. LSD did not produce noteworthy dose-dependent increases on MDPV-lever selection, and the highest dose tested, 0.1 mg/kg LSD (n = 7), only produced low partial substitution (28.80% ± 15.01). A RMANOVA failed to reveal a statistically significant effect of LSD on responses per second [$F(3, 21) = 2.40$, $p = .10$].

(+)-Fenfluramine. The percent MDPV-lever selection and response rates under (+)-fenfluramine (0.03 – 3 mg/kg) are displayed in Figure 6D. Percent MDPV-lever selection values remained low among the three lowest doses of fenfluramine tested (0.1 – 1 mg/kg), however 3 mg/kg fenfluramine produced full substitution in four of the eight rats (89.88 ± 10.12). A RMANOVA revealed a statistically significant effect of fenfluramine on responses per second [$F(5, 35) = 6.28$, $p = < .001$, $\eta^2 = 0.37$]. Dunnett's multiple comparisons tests revealed that the 0.03 and 3 mg/kg doses of fenfluramine each produced decreases in response rates compared to V.

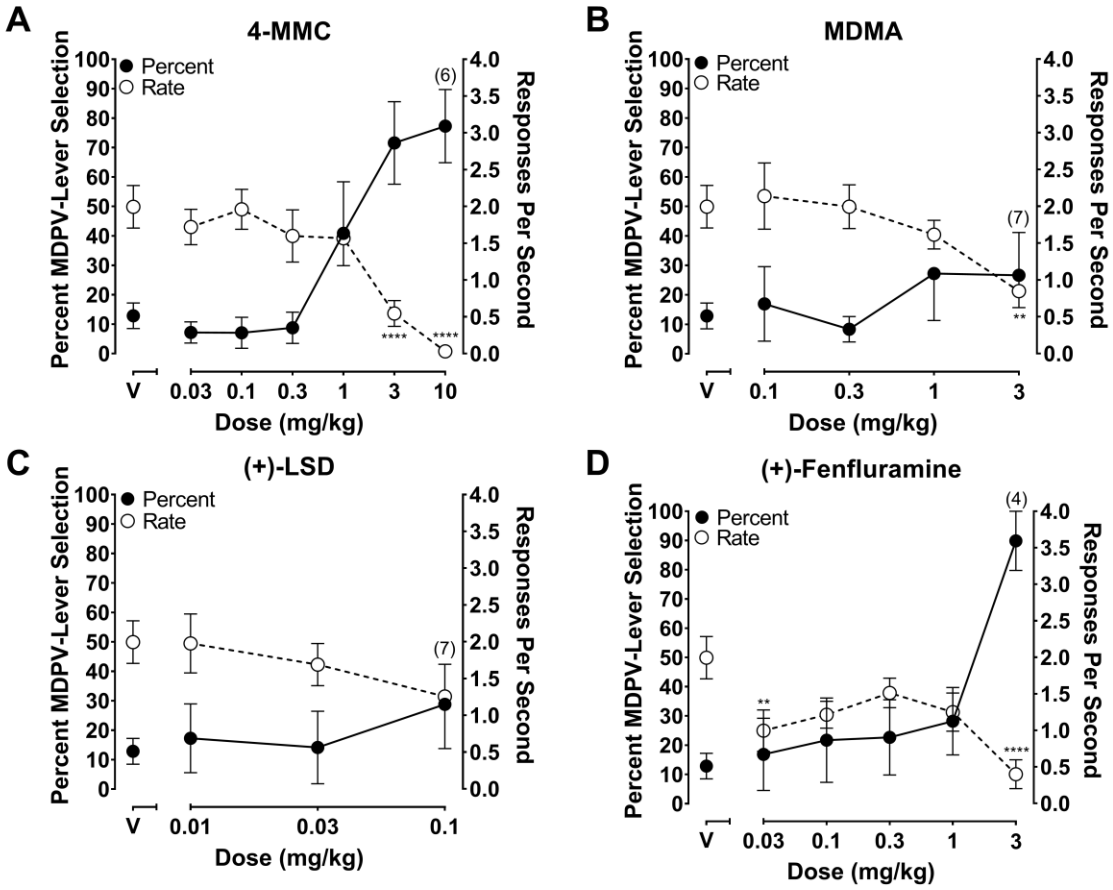

Figure 6. Results of substitution tests with doses of (A) 4-MMC, (B) MDMA, (C) (+)-LSD, and (D) (+)-fenfluramine in rats trained to discriminate 0.3 mg/kg MDPV from saline. Mean (±*SE*) percent MDPV-lever selection (left ordinate) and mean (±*SE*) responses per second (right ordinate). Rats that did not complete FR during a test session were not included in the percent drug-lever selection data (the number of rats is indicated in parenthesis, otherwise the number of rats was equal to 8). For the response rate data, statistical differences from V are indicated by asterisks (**$p < .01$ ****$p < .0001$).

Figure 7 presents a comparison between the foregoing graded dose-response curves for 4-MMC, MDMA, (+)-LSD, and (+)-fenfluramine to the same dose-response curves analyzed under a quantal approach, in rats trained to discriminate 0.3 mg/kg MDPV from saline. Similar to Figure 5 above, note the resemblance of the dose-response curves obtained between the percent MDPV-lever selection metric (graded) and the percent subjects selecting MDPV lever metric (quantal).

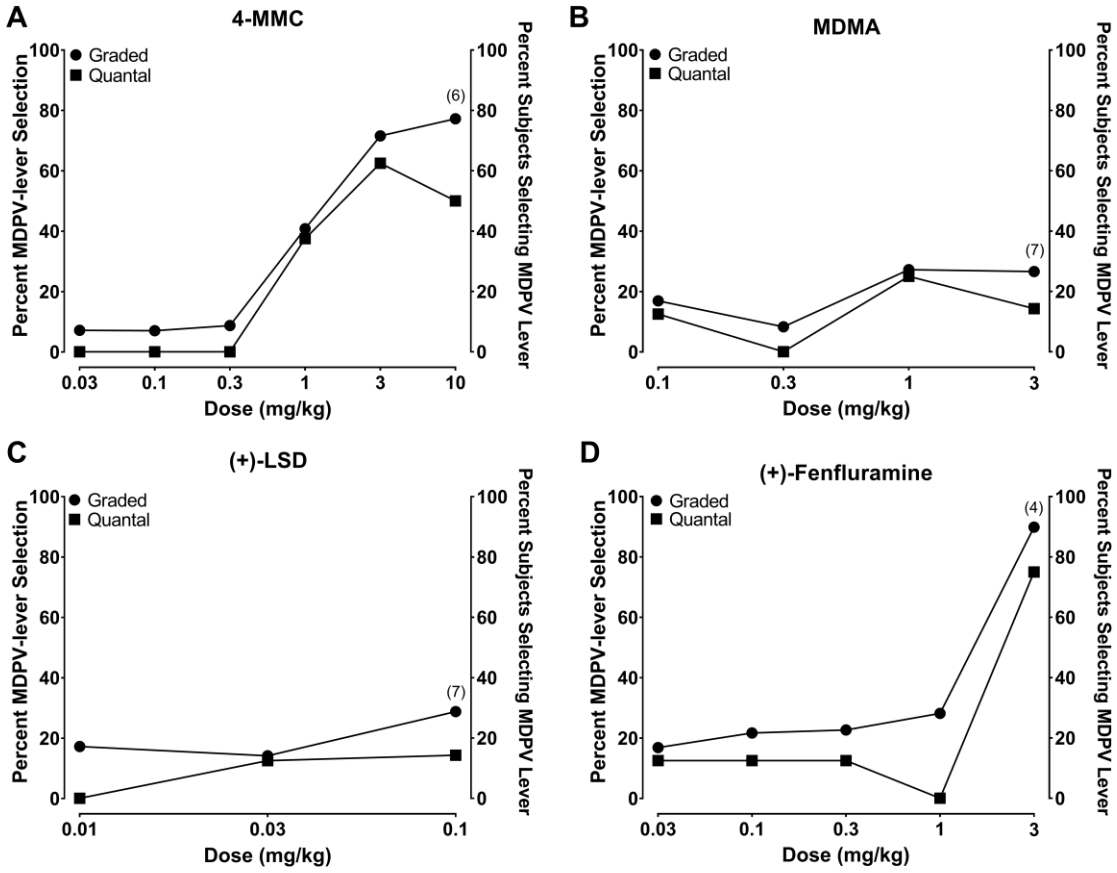

Figure 7. Quantal and graded results of substitution tests with doses of (A) 4-MMC, (B) MDMA, (C) (+)-LSD, and (D) (+)-fenfluramine in rats trained to discriminate 0.3 mg/kg MDPV from saline. Mean percent MDPV-lever selection (●, graded, left ordinate) and percent subjects selecting MDPV lever (■, quantal, right ordinate). Rats that did not complete FR during a test session were not included (the number of rats is indicated in parenthesis, otherwise the number of rats was equal to 8).

The foregoing substitution test results obtained in the 0.3 mg/kg MDPV-trained rats are summarized in Table 6 below.

Table 6

Summary of Substitution Test Data in 0.3 mg/kg MDPV Training Group

	Mean Maximum Percent Substitution ± *SE*	**ED_{50} (mg/kg)**	**95% CI (mg/kg)**
MDPV[a]	100 ± *0*	0.06	0.03 – 0.11[e]
4-MMC[b]	77.26 ± *12.46*	-----	-----
AMP[c]	100 ± *0*	0.14	0.08 – 0.24[e]
METH[d]	97.86 ± *2.14*	0.12	0.07 – 0.22[e]
COC[e]	97.92 ± *2.08*	0.73	0.37 – 1.47[acd]
MDMA[f]	27.24 ± *16.71*	-----	-----
LSD[g]	28.80 ± *15.01*	-----	-----
FEN[h]	89.88 ± *10.12**	-----	-----

A letter superscript indicates non-overlapping 95% CIs between two test compounds where applicable, and an asterisk superscript indicates that less than six subjects' substitution test results were used to calculate the associated mean maximum percent substitution value, precluding ED_{50} determination. Abbreviations: 3,4-methylenedioxypyrovalerone (MDPV), 4-methylmethcathinone (4-MMC), *d*-amphetamine (AMP), (+)-methamphetamine (METH), (-)-cocaine (COC), 3,4-methylenedioxymethamphetamine (MDMA), (+)-lysergic acid diethylamide (LSD), and (+)-fenfluramine (FEN).

1.0 mg/kg 4-MMC training group

4-MMC. The percent 4-MMC-lever selection and response rates under 4-MMC (0.01 - 1 mg/kg) are displayed in Figure 8A. 4-MMC dose-dependently increased 4-MMC-lever selection up to the 1.0 mg/kg training dose, which was the only dose to produce full substitution (96.19% ± 2.18) (ED_{50} = 0.18 mg/kg; 95% CI = 0.08 – 0.38 mg/kg). A one-way repeated-measures ANOVA (RMANOVA) failed to reveal a statistically significant effect of 4-MMC on responses per second [$F(5, 35) = 0.9917$, $p = .44$].

MDMA. The percent 4-MMC-lever selection and response rates under MDMA (0.01 - 1 mg/kg) are displayed in Figure 8B. MDMA dose-dependently increased 4-MMC-lever selection up to 1.0 mg/kg, which was the only dose to fully substitute for 1.0 mg/kg 4-MMC (96.34% ± 2.05) (ED_{50} = 0.16 mg/kg; 95% CI = 0.08 – 0.36 mg/kg). A RMANOVA failed to reveal a statistically significant effect of MDMA on responses per second [$F(4, 28) = 1.627$, $p = .20$].

(+)-LSD. The percent 4-MMC-lever selection and response rates under (+)-LSD (0.01 – 0.2 mg/kg) are displayed in Figure 8C. LSD dose-dependently increased 4-MMC-lever selection up to the 0.1 mg/kg LSD dose (49.22% ± 14.73) (n =7), but decreased 4-MMC-lever selection at the 0.2 mg/kg LSD dose (32.16% ± 3.13) (n = 2). A RMANOVA revealed a statistically significant effect of LSD on responses

per second [$F(4, 28) = 19.68$, $p = <.0001$, $\eta^2 = 0.66$]. Dunnett's multiple comparisons tests revealed that the 0.1 and 0.2 mg/kg doses of LSD produced decreases in response rate compared to V.

(+)-Fenfluramine. The percent 4-MMC-lever selection and response rates under (+)-fenfluramine (0.1 - 3 mg/kg) are displayed in Figure 8D. Fenfluramine dose-dependently increased 4-MMC-lever selection and produced partial substitution at 3 mg/kg (74.32% ± 15.67) (n =6). A RMANOVA revealed a statistically significant effect of fenfluramine on responses per second [$F(4, 28) = 9.12$, $p = <.0001$, $\eta^2 = 0.47$]. Dunnett's multiple comparisons tests revealed that the 0.3, 1, and 3 mg/kg doses of fenfluramine produced decreases in response rate compared to V.

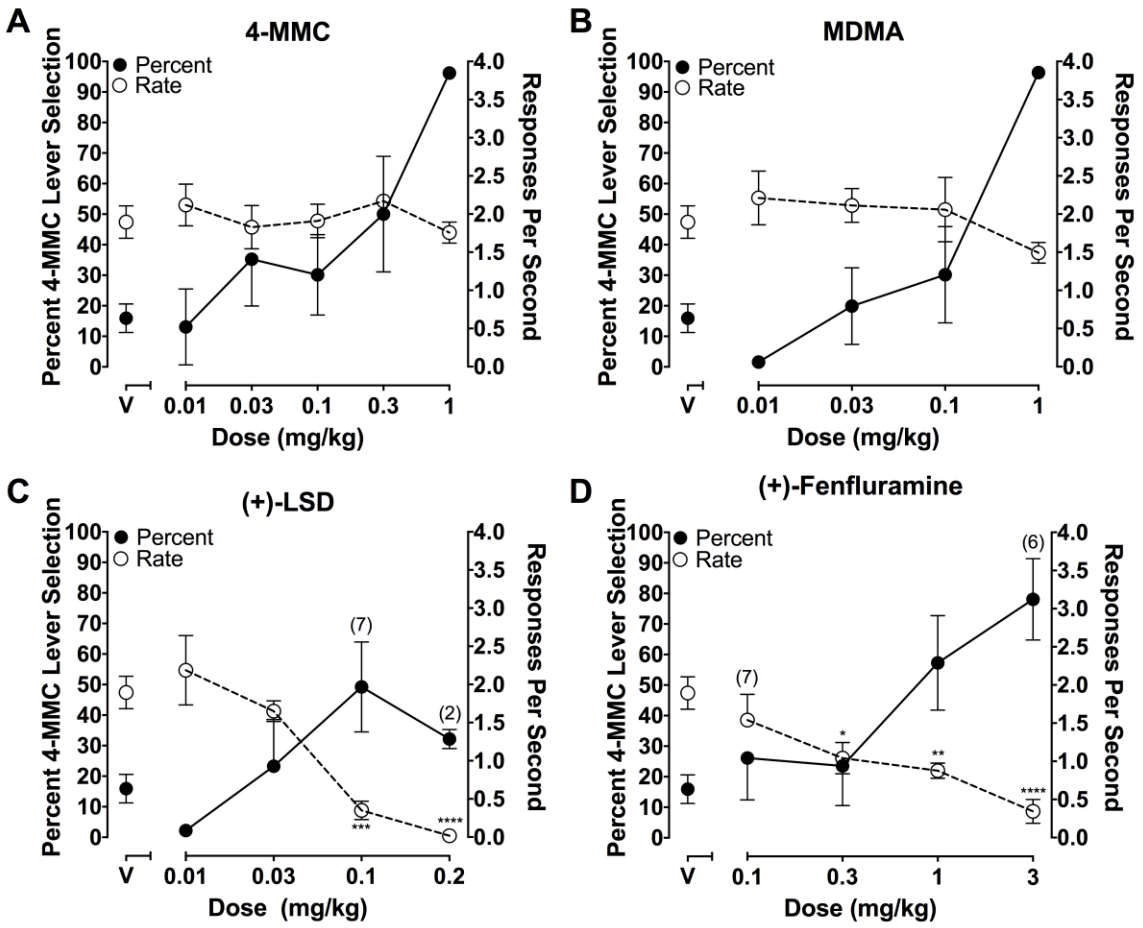

Figure 8. Results of substitution tests with doses of (A) 4-MMC, (B) MDMA, (C) (+)-LSD, and (D) (+)-fenfluramine in rats trained to discriminate 1.0 mg/kg 4-MMC from saline. Mean (±*SE*) percent MDPV-lever selection (left ordinate) and mean (±*SE*) responses per second (right ordinate). Rats that did not complete FR during a test session were not included in the percent drug-lever selection data (the number of rats is indicated in parenthesis, otherwise the number of rats was equal to 8). For the response rate data, statistical differences from V are indicated by asterisks (*p <.05, **p < .01, ***p <.001, ****p < .0001).

Figure 9 presents a comparison between the foregoing graded dose-response curves for 4-MMC, MDMA, (+)-LSD, and (+)-fenfluramine to the same dose-response curves analyzed under a quantal approach, in rats trained to discriminate 1.0 mg/kg 4-MMC from saline. Aside from the slight discrepancies in percent substitution among the 4-MMC and (+)-LSD curves, it appears that the graded and quantal approaches produced comparable dose-effect functions.

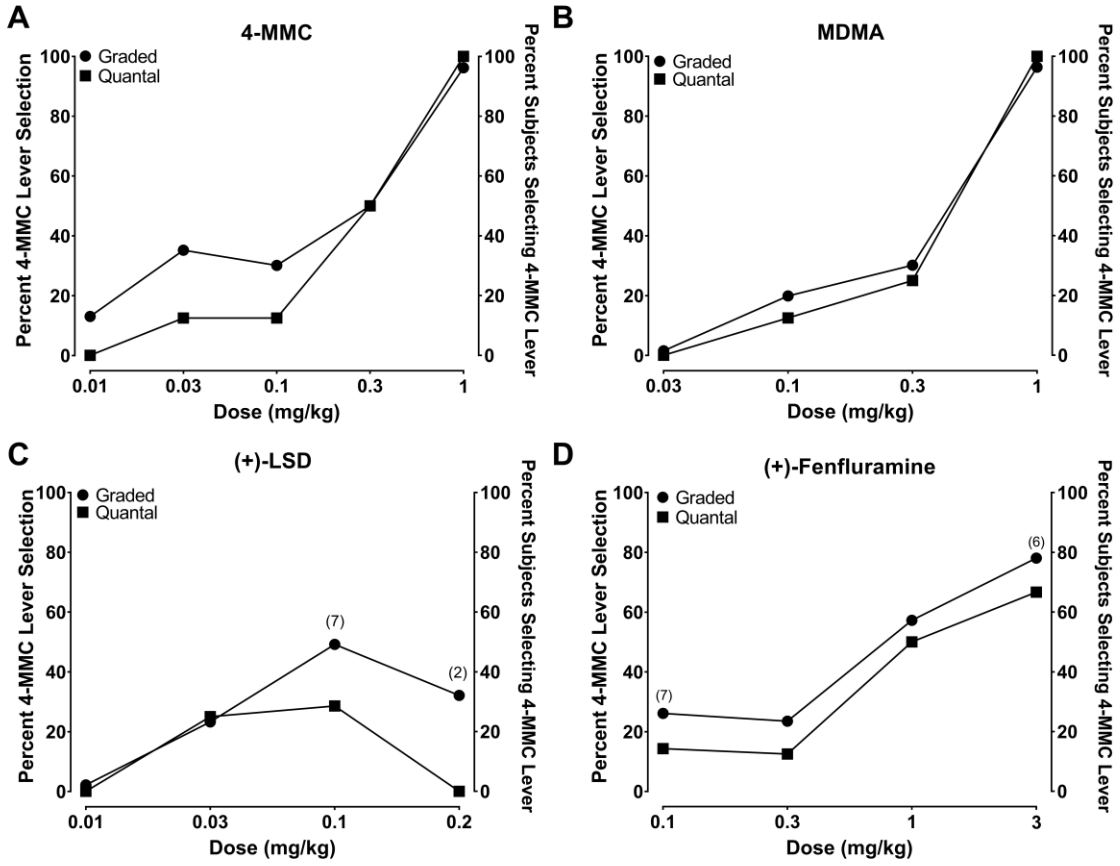

Figure 9. Quantal and graded results of substitution tests with doses of (A) 4-MMC, (B) MDMA, (C) (+)-LSD, and (D) (+)-fenfluramine in rats trained to discriminate 1.0 mg/kg 4-MMC from saline. Mean percent 4-MMC-lever selection (●, graded, left ordinate) and percent subjects selecting 4-MMC lever (■, quantal, right ordinate). Rats that did not complete FR during a test session were not included (the number of rats is indicated in parenthesis, otherwise the number of rats was equal to 8).

MDPV. The percent 4-MMC-lever selection and response rates under MDPV (0.03 – 3 mg/kg) are displayed in Figure 10A. MDPV dose-dependently increased 4-MMC-lever selection up to 3 mg/kg (n = 7), which was the only dose to fully substitute for 1.0 mg/kg 4-MMC (85.12% ± 13.49) (ED_{50} = 0.63 mg/kg; 95% CI = 0.31 – 1.30 mg/kg). A RMANOVA revealed a statistically significant effect of MDPV on responses per second [$F(5, 35) = 4.89$, $p = .0017$, $\eta^2 = 0.35$]. Dunnett's multiple comparisons tests revealed that the 1 and 3 mg/kg doses of MDPV produced decreases in response rate compared to V.

d-Amphetamine. The percent 4-MMC-lever selection and response rates under *d*-amphetamine (0.03 – 1 mg/kg) are displayed in Figure 10B. *d*-Amphetamine dose-dependently increased 4-MMC-lever selection up to the 1 mg/kg, which was the only dose to fully substitute for 1.0 mg/kg 4-MMC (81.47% ± 13.50) (ED_{50} = 0.48 mg/kg; 95% CI = 0.26 – 0.89 mg/kg). A RMANOVA failed to reveal a statistically significant effect of *d*-amphetamine on responses per second [$F(4, 28) = 2.67$, $p = .052$].

(+)-Methamphetamine. The percent 4-MMC-lever selection and response rates under (+)-methamphetamine (0.03 – 3 mg/kg) are displayed in Figure 10C. (+)-Methamphetamine dose-dependently increased 4-MMC-lever selection up to 3 mg/kg (n = 3), which was the only dose to fully substitute for 1.0 mg/kg 4-MMC (100% ± 0). An ED_{50} value was not computed for this test compound since only three out of the eight subjects responded under the highest tested dose. A RMANOVA revealed a statistically significant effect of responses per second [$F(5, 35) = 10.32$, $p < .0001$, $\eta^2 = 0.47$]. Dunnett's multiple comparisons tests revealed that the 3 mg/kg dose of methamphetamine produced decreases in responses per second compared to V.

(-)-Cocaine. The percent 4-MMC-lever selection and response rates under (-)-cocaine (0.03 - 10 mg/kg) are displayed in Figure 10D. Cocaine dose-dependently increased 4-MMC-lever selection up to 10 mg/kg, which was the only dose to fully substitute for 1.0 mg/kg 4-MMC (98.09% ± 1.36) (ED_{50} = 0.92 mg/kg; 95% CI = 0.34 – 2.08 mg/kg). A RMANOVA failed to reveal a statistically significant effect of cocaine on responses per second [$F(5, 35) = 1.81$, $p = .14$].

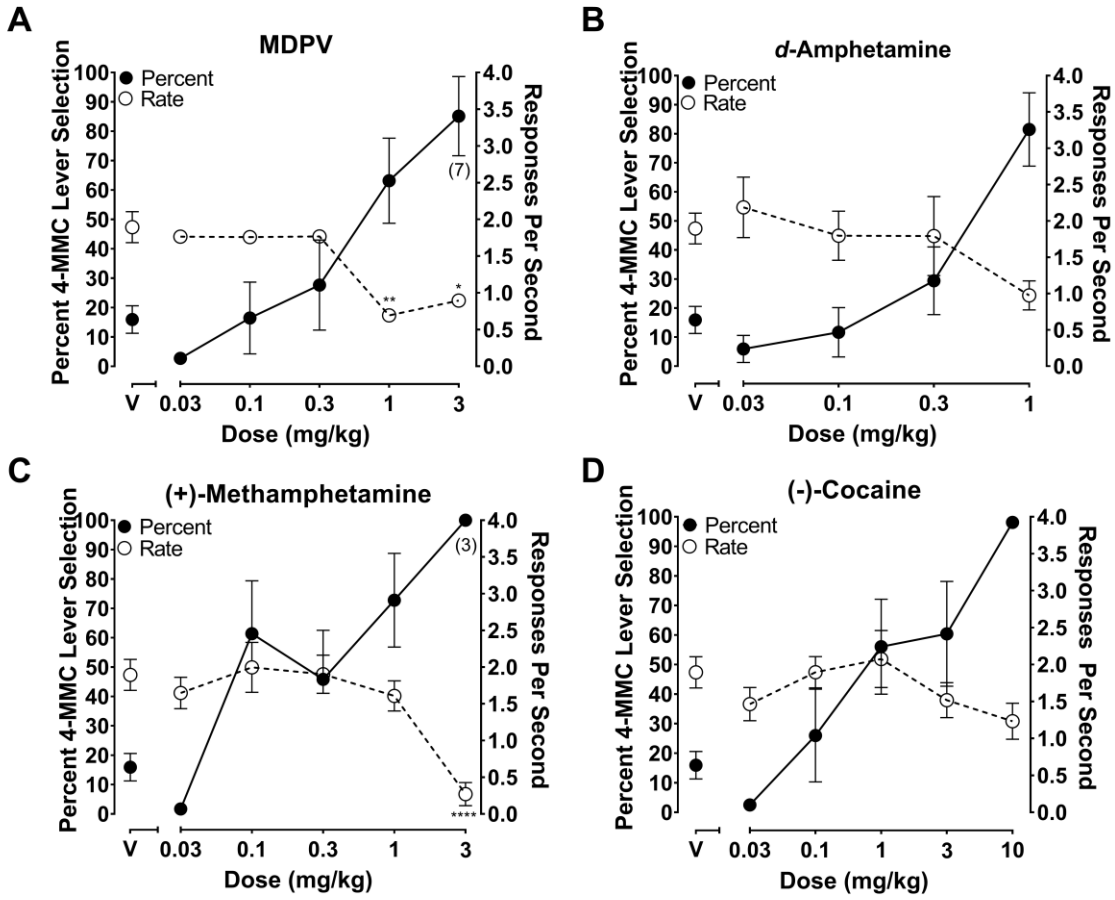

Figure 10. Results of substitution tests with doses of (A) MDPV, (B) *d*-amphetamine, (C) (+)-methamphetamine, and (D) (-)-cocaine in rats trained to discriminate 1.0 mg/kg 4-MMC from saline. Mean (±*SE*) percent 4-MMC-lever selection (left ordinate) and mean (±*SE*) responses per second (right ordinate). Rats that did not complete FR during a test session were not included in the percent drug-lever selection data (the number of rats is indicated in parenthesis, otherwise the number of rats was equal to 8). For the response rate data, statistical differences from V are indicated by asterisks (*$p < .05$; **$p < .01$; ****$p < .0001$).

Figure 11 presents a comparison between the foregoing graded dose-response curves for MDPV, *d*-amphetamine, (+)-methamphetamine, and (-)-cocaine to the same dose-response curves analyzed under a quantal approach, in rats trained to discriminate 1.0 mg/kg 4-MMC from saline.

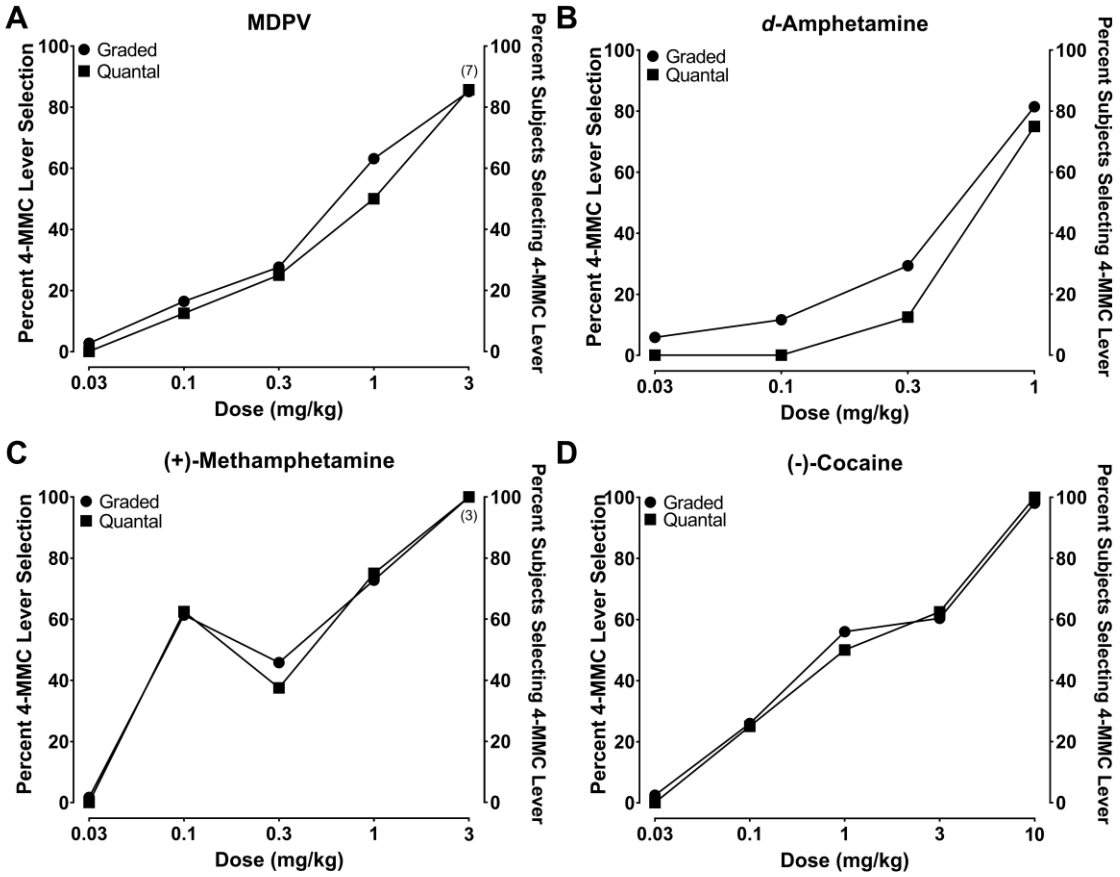

Figure 11. Quantal and graded results of substitution tests with doses of (A) MDPV, (B) *d*-amphetamine, (C) (+)-methamphetamine, and (D) (-)-cocaine in rats trained to discriminate 1.0 mg/kg 4-MMC from saline. Mean percent 4-MMC-lever selection (●, graded, left ordinate) and percent subjects selecting 4-MMC lever (■, quantal, right ordinate). Rats that did not complete FR during a test session were not included (the number of rats is indicated in parenthesis, otherwise the number of rats was equal to 8).

The foregoing substitution test results obtained in the 1.0 mg/kg 4-MMC trained rats are summarized in Table 7 below.

Table 7

Summary of Substitution Test Data in 1.0 mg/kg 4-MMC Group

	Mean Maximum Percent Substitution ± *SE*	**ED_{50} (mg/kg)**	**95% CI (mg/kg)**
4-MMC[a]	96.19 ± *2.18*	0.18	(0.08 – 0.38)
MDPV[b]	85.12 ± *13.49*	0.63	(0.31 – 1.30)
AMP[c]	81.47 ± *13.50*	0.48	(0.26 – 0.89)
METH[d]	100 ± *0**	-----	-----
COC[e]	98.09 ± *1.36*	0.92	(0.34 – 2.08)
MDMA[f]	96.34 ± *2.05*	0.16	(0.08 – 0.36)
LSD[g]	49.22 ± *14.73*	-----	-----
FEN[h]	78.08 ± *13.33*	-----	-----

An asterisk superscript indicates that less than six subjects' substitution test results were used to calculate the associated mean maximum percent substitution value, precluding ED_{50} determination. Abbreviations: 3,4-methylenedioxypyrovalerone (MDPV), 4-methylmethcathinone (4-MMC), *d*-amphetamine (AMP), (+)-methamphetamine (METH), (-)-cocaine (COC), 3,4-methylenedioxymethamphetamine (MDMA), (+)-lysergic acid diethylamide (LSD), and (+)-fenfluramine (FEN).

Relationship between 0.3 mg/kg MDPV and 1.0 mg/kg 4-MMC Cues

Figure 12 presents the mean maximum percent drug-lever selection values of the substitution test compounds and averaged vehicle test values obtained in the 0.3 mg/kg MDPV training group (see Table 6) plotted as a function of the same values obtained in the 1.0 mg/kg 4-MMC training group (see Table 7).

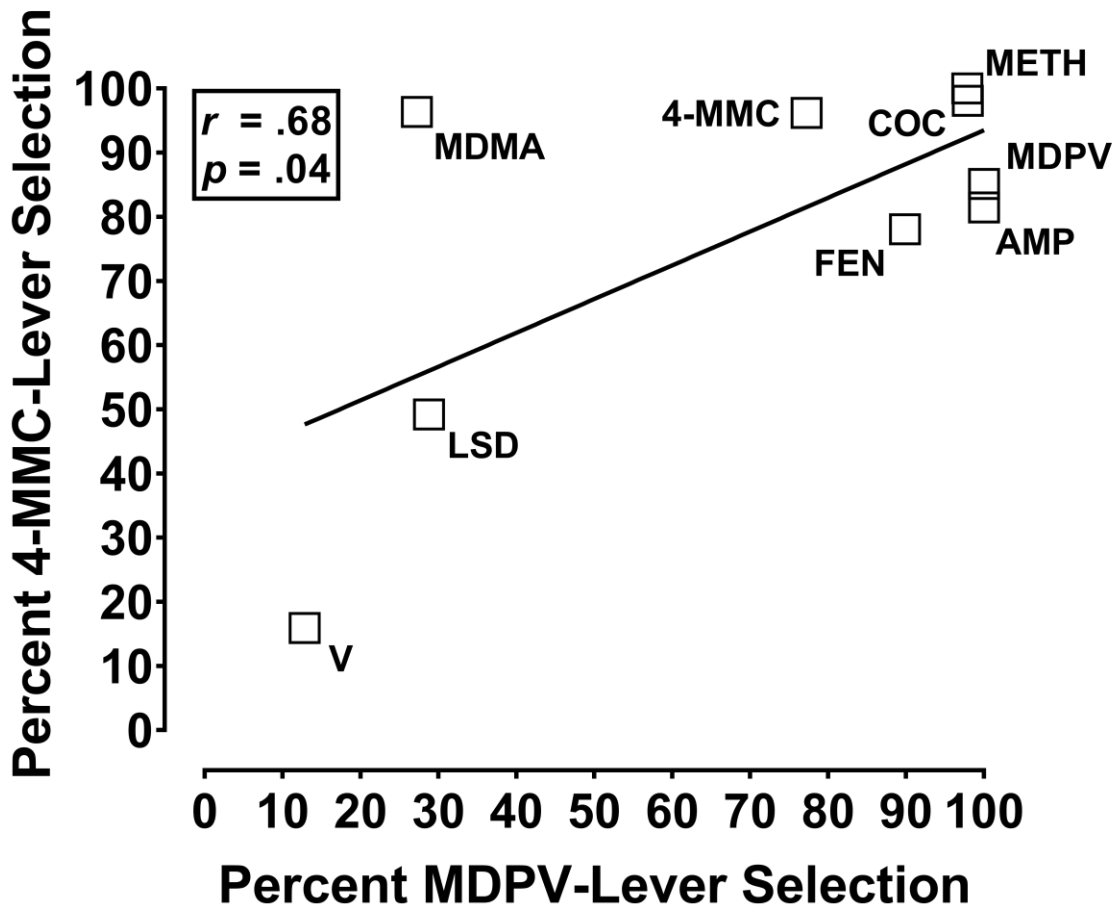

Figure 12. Percent drug-lever selection data from the present experiment are summarized for several substitution test compounds tested in one group of rats trained to discriminate 0.3 mg/kg MDPV from vehicle and in another group of rats trained to discriminate 1.0 mg/kg 4-MMC from vehicle. The highest percent of percent 4-MMC-lever selection is shown on the ordinate as a function of the highest percent MDPV-lever selection for each drug on the abscissa. A rectilinear regression line for the data and the Pearson *r* correlation coefficient are also displayed. Note: the sample sizes for each data point are consistent with the number of rats that completed the FR 20 for each substitution test compound. Abbreviations: V (vehicle), 3,4-methylenedioxypyrovalerone (MDPV), 4-methylmethcathinone (4-MMC), *d*-amphetamine (AMP), (+)-methamphetamine (METH), (-)-cocaine (COC), 3,4-methylenedioxymethamphetamine (MDMA), (+)-lysergic acid diethylamide (LSD), and (+)-fenfluramine (FEN).

DISCUSSION

Discriminative stimulus control by 0.3 mg/kg MDPV or 1.0 mg/kg 4-MMC was established within an average of ~35 and ~37 discrimination training sessions, respectively, although it is noteworthy that two of the subjects in the 4-MMC training group required remedial training sessions. These results demonstrate that 0.3 mg/kg MDPV and 1.0 mg/kg 4-MMC can serve as antecedent sources of control over lever-press responding in rats. As evidenced by comparable survival curves that display acquisition of discrimination as a function of training sessions, the selected training doses of MDPV and 4-MMC appear to have served as equieffective, salient discriminative cues under these testing conditions.

Inspection of individual subject performance in the percent drug-substitution measure (see Appendices A and B) reveals that in most testing sessions, subjects responded in an all-or-none fashion. That is, most subjects dose-dependently responded <20% or >80% under the substitution test compounds. Such responding is often considered characteristic of quantal performance—either the test drug cue resembles the training drug cue, or it does not. This type of responding can be contrasted with graded responding that is characterized by subjects' percent drug-lever selection values appearing proportional to increases in dose of a drug. Regardless of whether individual subject performance appears quantal, early behavioral research on conditional discrimination supports the notion that group or collated behavioral performances that appear graded are often the result of averaging individuals' quantal responses (e.g., Sidman, 1980; Bickel & Etzel, 1985).

Much of the discussion of graded versus quantal responding is due to interpretation issues associated with partial substitution percent drug-lever selection data (i.e., percent drug-lever selection values that fall between 20-80%). Previous reports have extensively discussed relationships between procedural variables (e.g., schedule of reinforcement, training dose) and relevant data outcomes, such as maximum discrimination accuracy and response rates (e.g., Overton, 1979; Stolerman & D'Mello, 1981; Stolerman, 1991). In light of the foregoing discussion on quantal and graded responding, it is noteworthy that previous research has demonstrated that the *type* of percent substitution data observed (i.e., graded or quantal) may be influenced by the programmed schedule of reinforcement. For example, McMillan, Li, and Hardwick (2001) trained pigeons to discriminate among 5 mg/kg pentobarbital, 5 mg/kg morphine, and saline under an FR 20 schedule or an FI 90-s schedule. The results of that study revealed that responding

under the FR 20 schedule generated quantal-like dose-response curves and those responding under the FI 90-s schedule generated graded-like dose-response curves (McMillan, Li, & Hardwick, 2001). Similar findings have been demonstrated elsewhere (e.g., Stolerman, 1989; Barrett, Caul, Huffman, & Smith, 1994; McMillan, Hardwick, & Li, 2001). In the present study, subjects were trained to respond under an FR 20 schedule of food reinforcement and, given that most recent DD studies completed in the WMU laboratory have included FR 20 schedules of food reinforcement, individual subjects have consistently displayed quantal-like responding under a variety of experimental conditions and substitution test compounds (Berquist and Baker, unpublished observations). Though beyond the scope of the present document to fully review the debate of quantal versus graded responding (for additional discussion, see Williams, 1987; Gauvin & Young, 1987; Mathis, Emmett-Oglesby, Harris, & Lal, 1987; Weiss & Schindler, 1987; Bickel, 1987; Barrett, Caul, Huffman, & Smith, 1994; Emmett-Oglesby, 1994; Gauvin & Holloway, 1994), some have argued that DD methods that generally yield quantal data ought to be abandoned (e.g., Overton, 1994), and others have used other procedures (e.g., conditioned taste aversion preparations) that more approximate graded responding (e.g., Mathis & Emmett-Oglesby, 1990; Riley et al., 1991).

Despite the theoretical disagreements that exist in interpreting partial percent drug substitution values, some evidence suggests that data path analyses, and thus determination of drug potency values, maximal effects (efficacy), etc., are not changed in any appreciable way under the graded and quantal methods. For example, Schechter (1997a) trained two separate groups of rats to discriminate 10 mg/kg cocaine or 2 mg/kg MDMA from saline. The training drugs were tested in each group of rats and the percent drug-lever selection values were separately analyzed as quantal and quantitative (graded) data. In both training groups, almost identical dose-response curves and ED_{50} values were obtained using the two data path analyses. As such, Schechter (1997a) suggested that analyses of quantal and graded data produce equivalent assessments of the percent drug-lever selection values. Direct comparisons of graded versus quantal dose-response determinations in the present study (see Figures 5, 7, 9, and 11) support this conclusion; although, some inconsistencies were observed at specific doses of test compounds in the present experiment. Similar comparisons and findings between quantal dose-response curves and graded dose-response curves obtained from subjects trained to discriminate 0.4 mg/kg *d*-amphetamine or 0.7 mg/kg norfenfluramine from saline have been reported as well (e.g., Schechter, 1997b). Moreover, in some

cases the schedule of reinforcement does not produce notable differences in stimulus generalization gradients, but may affect other variables included in the experiment, such as discrimination acquisition. For example, Kueh and Baker (2007) trained groups of rats to discriminate 1.5 mg/kg MDMA or 10 mg/kg cocaine from saline under an FR 20 or VI 15-s schedule of food reinforcement. The results of that study revealed negligible differences in percent drug-lever selection values of substitution test compounds between rats responding under the two schedules in each training drug group, however, the FR 20-trained rats in both groups acquired the discrimination more rapidly during training. These results are consistent with previous research demonstrating that schedule of reinforcement effects influence drug stimulus control (McMillan & Wenger, 1984; De Vry, Koek, & Slangen, 1984; Snodgrass & McMillan, 1991; Craft, Morgan, & Bernal, 1998).

The monoamine transporter blockers (MDPV, (-)-cocaine), DA releasers (*d*-amphetamine, methamphetamine), and a serotonin releaser ((+)-fenfluramine; in half of the sample) produced full substitution for the 0.3 mg/kg MDPV cue. Contrariwise, substrates for the monoamine transporters (4-MMC, MDMA) and a classic serotonergic hallucinogen ((+)-LSD) did not produce full substitution, although the 10 mg/kg 4-MMC dose produced a high partial substitution value (~78% 4-MMC-lever selection). These data are partially consistent with a previous study that trained mice to discriminate 0.3 mg/kg MDPV from saline (Fantegrossi, Gannon, Zimmerman, & Rice, 2013). Fantegrossi, Gannon, Zimmerman, and Rice (2013) reported in 0.3 mg/kg MDPV-trained mice that MDPV, methamphetamine, and MDMA produced full substitution, whereas the cannabinoid CB_1 and CB_2 receptor agonist, JWH-018, and an opioid agonist, morphine, failed to produce full substitution. There exist numerous differences between the present study and that of Fantegrossi et al. that may account for the discrepant substitution profile with MDMA. Specifically, the present study trained Sprague-Dawley rats to discriminate 0.3 mg/kg MDPV from saline under an FR 20 schedule of food reinforcement, with a 15-min pre-session injection interval, and included discrete-dosing, extinction sessions to test the substitution test compounds. Contrariwise, Fantegrossi et al. trained NIH Swiss mice to discriminate 0.3 mg/kg MDPV from saline under an FR 10 -10-s time-out schedule of sweetened milk reinforcement, with a 10-min pre-session injection interval, and used cumulative-dosing, extinction sessions to test the substitution test compounds.

As mentioned, only a single report has been published that included a dose of MDPV as the training drug in a DD procedure (Fantegrossi, Gannon, Zimmerman, & Rice, 2013), precluding thorough comparisons of the results obtained in the present study. Nevertheless, because MDPV is pharmacologically similar to cocaine, albeit more potent, it is possible that MDPV may share a similar substitution profile to MDMA, comparable to the similarities between cocaine and MDMA. For example, Khorana, Pullagurla, Young, and Glennon (2004) reported that in Sprague-Dawley rats trained to discriminate 1.5 mg/kg MDMA from saline, cocaine fully substituted for the MDMA cue. In contrast, in Sprague-Dawley rats trained to discriminate 8 mg/kg cocaine from saline, racemic MDMA, *S*(+)-MDMA, and *R*(-)-MDMA all failed to produce complete generalization. These findings indicate the substitution profile between MDMA and cocaine is asymmetrical. In contrast, Broadbent, Michael, and Appel (1989) trained groups of rats to discriminate 3.5, 10, or 20 mg/kg cocaine from saline, and found that the (*l*)-MDMA isomer only generalized in 3 out of 5 of the 20 mg/kg cocaine-trained rats. Thus, the training dose of cocaine likely influences subsequent percent drug-lever selection values with substrates for monoamine transporters (also, see Schechter, 1997c). It is possible that MDMA would produce complete generalization in rats trained to discriminate a different dose of MDPV than the 0.3 mg/kg MDPV dose used in the present experiment, as previous research suggests that the MDMA cue may become more dopaminergic-mediated as the dose increases (e.g., Harper, Langen, & Schenk, 2014).

Harvey and Baker (2016) trained rats to discriminate 1.5 mg/kg MDMA or a 0.5 mg/kg *d*-amphetamine + 1.5 mg/kg MDMA mixture. Interestingly, cocaine, *d*-amphetamine, and MDPV produced full substitution for the *d*-amphetamine + MDMA cue, but not for the MDMA-only cue, whereas MDMA and 4-MMC fully substituted in for both training cues. These findings suggest that the DA-releasing effects of 0.5 mg/kg *d*-amphetamine added to the 1.5 mg/kg MDMA cue were necessary for subjects to display complete stimulus generalization when tested with monoamine transporter blockers and a DA releaser. In contrast, *d*-amphetamine, methamphetamine (in 3 out of 8 subjects), MDPV, and cocaine produced full substitution in 1 mg/kg 4-MMC-trained rats in the present study. Although previous reports have demonstrated that 4-MMC produces neurochemical effects that are similar to MDMA (e.g., Kehr et al. 2011; Baumann et al., 2012; Pfil, Reither, & Hornykiewicz, 2015), it is presently unclear why the aforementioned drugs fully substituted in the 1 mg/kg 4-MMC-trained subjects, but *d*-amphetamine and

cocaine did not produce full substitution in the 1.5 mg/kg MDMA-trained subjects used in the study reported by Harvey and Baker (2016). Possible reasons for such findings may be that, similar to the foregoing relationship between MDMA and cocaine, substitution effects may be asymmetrical between MDMA and 4-MMC, or a different training dose of MDMA would be necessary for DA releasers and monoamine transporter blockers to fully substitute.

In addition to a potential asymmetric substitution profile between MDPV and MDMA, the discrepant substitution results with MDMA in the present study compared to that reported by Fantegrossi, Gannon, Zimmerman, and Rice (2013) could be due to different testing procedures. Specifically, Fantegrossi et al. used cumulative-dosing testing sessions and the present study used discrete-dosing testing sessions. However, previous research directly comparing cumulative-dosing testing to discrete-dosing session testing suggests that it is unlikely that the discrepant MDMA substitution tests results are due to these procedural differences. For example, Schechter (1997a) trained groups of rats to discriminate 10 mg/kg cocaine from saline or 2 mg/kg MDMA from saline. Subgroups within each of the training groups were exposed to cumulative-dosing testing procedures or discrete-dosing testing sessions. The results of that study revealed no difference in the training drugs' dose-response curves or ED_{50} values.

As a final point, species differences may account for the variant effects of MDMA on MDPV percent-lever selection between the present study and that of Fantegrossi, Gannon, Zimmerman, and Rice (2013), however, additional DD studies directly comparing the species under identical procedures would be necessary to evaluate this speculation. There are currently no known DD studies that have directly compared the generalization gradients determined from mice and rats, although it is worth noting that *S*(+)-amphetamine and cocaine fully-substituted for a 1.5 mg/kg *S*(+)-MDMA cue in mice (effects that are similar to the foregoing DD studies using rats), but not for a 1.5 mg/kg *R*(-)-MDMA cue in a different group of mice (Murane, Murai, Howell, & Fantegrossi, 2009), suggesting apparent differences in interoceptive effects elicited by MDMA enantiomers. Moreover, previous research has observed comparatively greater neurotoxic effects of MDMA on dopaminergic functioning in mice relative to rats, and greater neurotoxic effects of MDMA on serotonin functioning in rats relative to mice (for review, Easton & Marsden, 2006). Thus, it is possible that drugs that increase extracellular monoamine levels may differentially affect discriminative stimulus effects between mice and rats.

Despite the discrepant substitution results observed with MDMA between the present study and the Fantegrossi, Gannon, Zimmerman, and Rice (2013) study, MDPV and methamphetamine displayed full substitution in both studies. Moreover, the ED_{50} values are somewhat comparable between these studies (present study ED_{50} values, expressed in mg/kg: MDPV = 0.06; methamphetamine = 0.12; Fantegrossi et al. (2013) ED_{50} values: MDPV = 0.03; methamphetamine = 0.08); although, for both MDPV and methamphetamine in Fantegrossi et al., the observed potency values were lower. Such differences between the estimated ED_{50} values may be due to the aforementioned species differences (i.e., mice may be more sensitive than rats to the effects of increased extracellular dopamine).

As mentioned, MDMA and 4-MMC failed to produce complete generalization in 0.3 mg/kg MDPV-trained rats in the present study, however 4-MMC approached full substitution at the highest dose tested. Although only a single DD study has been published using a dose of MDPV as the training drug (Fantegrossi, Gannon, Zimmerman, & Rice, 2013), other studies have tested 4-MMC for substitution in rats trained to discriminate a DA releaser, methamphetamine, or a monoamine transporter blocker, cocaine, from saline. For example, Gatch, Taylor, and Forster (2013) trained groups of rats to discriminate 1 mg/kg methamphetamine or 10 mg/kg cocaine from saline and tested 4-MMC for substitution. In that study, 4-MMC equipotently produced full substitution for both training drug cues. It is noteworthy that Gatch et al. used an approximately 33 fold higher dose of cocaine (10 mg/kg) for training than the dose used for MDPV (0.3 mg/kg) in the present study. Previous research has demonstrated that MDPV is ~10 times more potent than cocaine at producing locomotor activation (e.g., Baumann et al., 2013), comparison of the ED_{50} values of MDPV (ED_{50} = 0.06 mg/kg) and (-)-cocaine (ED_{50} = 0.73 mg/kg) obtained in the present study reveals that MDPV is approximately 12 times more potent than (-)-cocaine at producing the 0.3 mg/kg MDPV cue, a relative potency difference that appears consistent with that reported by Baumann et al. (2013). In any event, future studies including a higher training dose of MDPV may be necessary for substrates at monoamine transporters, such as MDMA and 4-MMC, to fully substitute in male rats.

As mentioned, four of eight subjects in the 0.3 mg/kg MDPV group displayed complete generalization when tested with 3 mg/kg (+)-fenfluramine. Schechter and Rosecrans (1973) reported that fenfluramine failed to fully substitute for a 10 mg/kg *d*-amphetamine cue in rats. Similarly, Goudie (1977) reported that in female rats trained to discriminate 3 mg/kg fenfluramine from saline, *d*-amphetamine failed

to fully substitute for the fenfluramine cue. In addition, a later study including human volunteers who were trained to discriminate 10 mg *d*-amphetamine from placebo observed that 20 and 40 mg fenfluramine failed to fully substitute for the *d*-amphetamine cue (Chait, Uhlenhuth, & Johanson, 1986). These findings suggest that the dopamine releasing effects of amphetamine produce interoceptive cues that are dissimilar from fenfluramine's interoceptive cues (also see White & Appel, 1981). Later studies revealed that fenfluramine's interoceptive cues are likely mediated by serotonergic, as opposed to dopaminergic, mechanisms. For example, McElroy and Feldman (1984) reported that in rats trained to discriminate 3 mg/kg fenfluramine from saline, *p*-chloroamphetamine, *p*-fluroamphetamine, and norfenfluramine fully substituted for the fenfluramine cue. Contrariwise, fluoxetine, methysergide, cinanserin, chlordiazepoxide, and 5-hydroxytryptophan failed to fully substitute for the fenfluramine cue, although 5-hydroxytryptophan produced 56% drug-appropriate responding. Also relevant to the present study, McElroy and Feldman (1984) observed that the (+)-fenfluramine isomer more potently substituted for the fenfluramine cue than the (-)-fenfluramine isomer, suggesting that the former isomer more potently affects serotonergic mechanisms, an effect consistent with previous *in vitro* experiments (e.g., Buczko, De Gaetano, & Garattini, 1975). Finally, McCreary, Filip, and Cunningham (2003) reported that in rats trained to discriminate 1 mg/kg fenfluramine from saline, a 5-$HT_{2C/1B}$ receptor agonist (mCPP) and a 5-HT_{2C} receptor agonist (MK 212) fully substituted for the fenfluramine cue. Moreover, a 5-HT_{2C} receptor antagonist, SB 206553, dose-dependently reduced drug-appropriate responding. The results of that report suggest that the stimulation of the 5-HT_{2C} receptor elicits the 1 mg/kg fenfluramine cue. Considered together, the foregoing studies implicate the role of serotonergic mechanisms in mediating fenfluramine's discriminative cue. Given that MDPV produces increases in extracellular levels of dopamine and norepinephrine, with comparatively weaker effects on serotonin (e.g., Baumann et al., 2013), it is surprising that 3 mg/kg fenfluramine fully substituted for 0.3 mg/kg MDPV in half the subjects. It is presently unknown if MDPV's discriminative effects (including downstream effects) are mediated by stimulation of serotonergic mechanisms, although a recent report revealed that the rewarding and locomotor-stimulant effects of cocaine (a drug with pharmacological actions similar to MDPV) are attenuated by 5-HT_{2C} receptor stimulation (Craige & Unterwald, 2013). Thus, it is possible that the interoceptive effects of MDPV may

involve serotonergic mechanisms. Further drug discrimination research including drugs with selective actions on serotonergic targets is warranted to evaluate this possibility.

An additional explanation for the unexpected substitution profile of (+)-fenfluramine may be the subjects' extensive drug and training histories by the time this drug was assessed for substitution. Drugs with variable pharmacological effects were used in the present study to provide a comprehensive substitution profile of MDPV. Although high doses of the substitution test compounds were avoided to prevent potential neurotoxicity, it is possible that the subjects' drug history influenced substitution test results. It is noteworthy that (+)-fenfluramine, in particular, was the last tested drug in the present study. Baker and Makhay (1996) demonstrated that (+)-fenfluramine injections altered the substitution profile in 1.5 mg/kg MDMA-trained rats, supporting the notion that the drug discrimination assay is sensitive to intermittent drug-dosing regimens. In contrast, previous research has demonstrated that a subject's drug training history may not affect drug stimulus control in the context of phenobarbital discrimination in pigeons, although phenobarbital stimulus control may weaken in the absence of training (e.g., McMillan, Sun, & Hardwick, 1996). However, it is presently uncertain if psychostimulant stimulus control decays following extended exposure to drugs of different pharmacological classes. It is therefore possible, though presently unknown, if the drug history of 0.3 mg/kg MDPV-trained rats altered sensitivity to the stimulus effects of compounds tested later in this study. Assessment of the impact of drug history on generalization gradients using drug discrimination procedures may be a viable research pursuit.

Only one published study has trained rats to discriminate the interoceptive effects of 4-MMC from saline. Varner et al. (2013) trained male Long-Evans hooded rats to discriminate 3.2 mg/kg 4-MMC from saline under an FR 20 schedule of food reinforcement. In that study, only 3.2 mg/kg 4-MMC and 3.2 mg/kg MDMA produced full substitution. Although ED_{50} values were not provided for 4-MMC and MDMA by Varner et al., visual inspection of dose response curves in their report indicates possible ED_{50} values for 4-MMC of ~1.0 mg/kg and for MDMA ~1.5 mg/kg (present study ED_{50} values: 4-MMC = 0.18 mg/kg; MDMA = 0.16 mg/kg) . Thus, as expected, the 1 mg/kg 4-MMC-trained rats in the present study appear more sensitive to the interoceptive effects of these substances compared to those rats trained to discriminate 3.2 mg/kg 4-MMC in the Varner et al. study. In addition, Varner et al. reported that 18 mg/kg cocaine and 1.0 mg/kg methamphetamine, approached full substitution with 75.86%- and 72.86% 4-MMC-lever

selection, respectively, whereas higher doses produced reductions in percent 4-MMC-lever responding. In the present study, 1 mg/kg 4-MMC, 1 mg/kg MDMA, 3 mg/kg (+)-methamphetamine (n = 3), and 10 mg/kg (-)-cocaine produced full substitution in 4-MMC-trained rats. Notwithstanding the procedural and rat strain differences between the present study and that by Varner et al., the training dose of 4-MMC is likely to play a major role in the generalization gradients. In the study by Varner et al., relatively higher doses of DA releasers approached full substitution before producing behavioral disruption. Similar findings have been observed in 3 mg/kg 4-MMC-trained rats (Berquist, Thompson, & Baker, unpublished observations). Contrariwise, it seems as though the 1 mg/kg 4-MMC-trained rats in the present study are comparatively less sensitive to the disruptive effects of DA releasers, although (+)-methamphetamine only fully substituted in three out of eight subjects.

It is likely that differences in substitution profiles obtained in the present study and that of Varner et al. (2013) are due to different 4-MMC training doses. However, other procedural differences should also be noted between these studies. The present study assessed substitution under extinction, with individual test doses assessed on separate occasions. Varner et al. (2013) used cumulative-dosing procedures and responses were reinforced during test sessions. In one study, Kaempf and Kallman (1987) trained rats to discriminate 3 mg/kg morphine from saline, and then tested the training drug on two occasions (1-5 mg/kg morphine) for stimulus substitution under 4-min reinforced and non-reinforced conditions. Kaempf and Kallman reported statistical differences in the generalization gradients between the reinforced and non-reinforced conditions. It is worth noting that Kaempf and Kallman did not end the session after a subject completed first FR 32 during the testing conditions; rather, a subject was permitted to respond (and receive reinforcers in the reinforced condition) for the entire 4-min session. Unfortunately, there are currently no known published studies that have directly compared reinforced test sessions to extinction test sessions *and* ended the test session following completion of the first response requirement. Without direct comparisons of reinforced and extinction sessions ending after the first response in the same study, it remains undetermined whether reinforced test sessions produce changes in generalization gradients obtained using DD procedures.

As a final point, it is worth noting that although direct comparisons between substitution profiles generated from 0.3 mg/kg MDPV- and 1 mg/kg 4-MMC-trained rats was not the purpose of the present

study, drug-lever selection by subjects that displayed high percent substitution values for some test compound in one training group was moderately correlated (except for MDMA) with drug-lever selection by subjects in the other training group (see Figure 12). As such, these findings support previous reports indicating that MDPV and 4-MMC can produce comparable subjective effects in humans (e.g., Ross, Reisfield, Watson, Chronister, & Goldberger, 2012). Moreover, to further characterize the interoceptive effects produced by MDPV and 4-MMC, future studies should include different training doses of the drugs, and blockade tests with drugs possessing selective receptor affinities to determine the neural mechanisms involved in mediating their discriminative cues.

The DD assay serves as a highly valuable investigative tool in behavioral pharmacology and neurobiological research. Numerous studies have demonstrated that nonhuman subjects and human volunteers trained to discriminate a dose of a drug from vehicle, doses of the same drug, doses of other drugs, or drug mixtures are sensitive to the effects of test drugs with comparable interoceptive effects. Moreover, rigorous investigations into neural mechanisms responsible for mediating such effects serves as one of the DD paradigm's most appealing qualities, especially because such experimentation is often precluded in human volunteers. The research included herein serves to perpetuate the utility of the DD model as a predictive and useful drug-detection assay.

Conclusion

Synthetic cathinones are currently an international public health concern given their untoward effects on personal and social harm measures. Through continued scientific investigations of their behavioral and neurochemical effects, we can better understand their abuse potential and eventually develop treatment strategies for users who consume these drugs to excess. Toward this goal, characterization of the psychopharmacology of synthetic cathinones remains a socially-relevant scientific endeavor. The research included herein serves as one step toward achieving that goal.

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APPENDIX A

Individual Subject Substitution Test and Response Rate Results in 0.3 mg/kg MDPV Training Group

Table 1

Individual Subject Performances for MDPV Substitution Tests and Response Rates in 0.3 mg/kg MDPV-trained Rats

Percent MDPV-Lever Selection								
	Subjects							
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0	100	0	0	0	18.18	0	0	26.67
0.01	0	0	100	0	12.5	0	0	0
0.03	27.59	19.23	100	0	12.5	0	8	0
0.1	100	100	100	0	0	57.41	29.41	47.92
0.3	100	100	100	100	100	100	100	100
Response Rate								
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0	1.35	1.96	2.94	0.95	1.23	2.38	1.89	1.18
0.01	2.86	6.06	2.44	1.54	1.67	2.67	1.72	1.80
0.03	2.25	1.60	2.82	1.67	1.21	2.22	2.41	1.07
0.1	1.32	1.94	1.79	1.65	1.20	2.23	1.66	1.75
0.3	1.60	2.08	2.17	0.41	0.91	1.85	1.92	1.79

Table 2

Individual Subject Performances for 4-MMC Substitution Tests and Response Rates in 0.3 mg/kg MDPV- trained Rats

Percent MDPV-Lever Selection								
	Subjects							
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0.03	0	24.14	0	0	6.62	4.55	0	22.22
0.1	43.24	8.70	0	0	4.55	0	0	0
0.3	0	8.70	0	8.33	0	8.70	0	44.44
1	100	4.55	100	0	22.22	0	0	100
3	16	100	4.55	67.31	100	100	100	84.62
10	*DNC*	100	20.45	*DNC*	100	95.65	69.70	77.78

DNC = did not complete FR 20

Response Rate								
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0.03	1.72	1.56	2.86	0.75	1.29	1.80	2.50	1.28
0.1	1.65	3.03	2.06	2.30	1.11	2.86	1.83	0.85
0.3	1.39	3.77	1.25	0.93	0.63	2.07	1.87	0.89
1	0.93	3.73	1.90	1.16	0.64	1.80	1.96	0.40
3	0.20	1.10	1.38	0.16	0.25	0.20	0.88	0.20
10	0.00	0.08	0.04	0.00	0.03	0.02	0.04	0.04

Table 3

Individual Subject Performances for d-Amphetamine Substitution Tests and Response Rate in 0.3 mg/kg MDPV-trained Rats

Percent MDPV-Lever Selection								
	Subjects							
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0	0	4.55	4.55	8.33	41.67	4.55	0	32.26
0.03	0	0	0	0	4.55	0	0	50
0.1	100	12.5	12	4.17	29.03	0	0	100
0.3	100	100	43.90	95.24	4.55	100	100	100
1	100	100	100	100	100	100	100	100
Response Rate								
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0	2.33	2.72	1.49	1.85	1.34	2.34	2.33	1.86
0.03	1.54	7.14	2.53	3.70	1.30	2.63	1.61	1.63
0.1	1.61	4.07	1.16	1.74	1.38	2.04	2.50	1.09
0.3	2.30	2.25	0.90	1.08	1.09	2.06	2.06	1.32
1	2.50	1.80	1.43	0.85	0.94	2.20	1.87	1.75

Table 4

Individual Subject Performances for (+)-Methamphetamine Substitution Tests and Response Rates in 0.3 mg/kg MDPV-trained Rats

Percent MDPV-Lever Selection								
	Subjects							
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0.03	100	0	0	4.55	8.70	0	0	0
0.1	43.24	100	0	96	8.70	0	0	0
0.3	8.70	100	100	92	100	100	100	94.29
1	100	100	100	82.86	100	100	100	100
Response Rate								
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0.03	2.17	6.45	2.53	2.34	1.50	2.53	1.57	1.87
0.1	1.95	3.03	2.15	1.14	1.00	2.11	1.57	1.82
0.3	2.19	3.70	0.77	0.83	1.98	1.69	1.12	0.99
1	1.90	2.70	1.20	0.77	2.06	1.45	1.82	2.86

Table 5

Individual Subject Performances for (-)-Cocaine Substitution Tests and Response Rates in 0.3 mg/kg MDPV-trained Rats

Percent MDPV-Lever Selection								
	Subjects							
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0.1	0	0	0	0	8.70	40.38	0	0
0.3	100	100	0	4.55	4.55	51.28	0	0
1	100	100	7.69	0	100	4.55	100	100
3	44.74	0	100	3.45	100	100	100	95.65
10	100	100	100	83.33	100	100	100	100
Response Rate								
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0.1	3.28	3.85	1.97	1.71	1.49	2.30	1.12	2.67
0.3	1.68	4.08	1.75	3.38	1.39	1.92	1.65	1.85
1	0.92	2.33	1.07	1.90	1.57	1.83	2.35	0.56
3	1.50	5.71	2.06	1.26	1.69	1.89	1.71	0.77
10	2.30	2.78	1.20	1.55	2.13	1.83	1.36	2.56

Table 6

Individual Subject Performances for MDMA Substitution Tests and Response Rates in 0.3 mg/kg MDPV-trained Rats

Percent MDPV-Lever Selection								
	Subjects							
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0	0	0	0	10.87	59.26	0	0	0
0.1	100	0	0	0	35.29	0	0	0
0.3	0	4.55	0	4.55	18.52	0	34.38	4.55
1	0	5.41	0	0	100	0	12.5	100
3	36.36	0	0	50	100	0	0	*DNC*

DNC = did not complete FR 20

Response Rate								
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0	3.17	7.14	2.56	0.67	2.50	1.96	1.34	1.60
0.1	1.54	5.26	1.92	1.82	1.81	1.55	1.79	1.42
0.3	2.22	3.44	2.82	1.47	2.11	1.67	1.43	0.81
1	1.30	1.34	2.25	2.11	0.98	2.38	1.50	1.06
3	0.67	1.85	0.73	0.13	0.71	1.44	1.26	0

Table 7

Individual Subject Performances for (+)-LSD Substitution Tests and Response Rates in 0.3 mg/kg MDPV-trained Rats

Percent MDPV-Lever Selection								
	Subjects							
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0.01	0	0	0	0	16	0	26.67	95.24
0.03	100	0	4.55	0	8.70	0	0	0
0.1	0	0	58.33	*DNC*	100	43.24	0	0
DNC = did not complete FR 20								
Response Rate								
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0.01	2.44	3.57	0.95	3.13	1.80	2.25	1.62	0.07
0.03	1.19	2.78	1.25	0.66	1.85	2.94	1.69	1.14
0.1	1.74	4.00	0.73	0.00	1.04	1.46	0.67	0.41

Table 8

Individual Subject Performances for (+)-Fenfluramine Substitution Tests and Response Rates in 0.3 mg/kg MDPV-trained Rats

Percent MDPV-Lever Selection								
	Subjects							
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0.03	27.59	0	0	0	100	7.32	0	0
0.1	73.53	0	0	0	100	0	0	0
0.3	0	47.62	0	0	33.82	0	0	100
1	0	68.75	0	28.43	67.74	0	60.61	0
3	59.52	100	*DNC*	*DNC*	100	100	*DNC*	*DNC*

DNC = did not complete FR 20

Response Rate								
Dose (mg/kg)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
0.03	0.81	1.23	2.15	0.40	0.09	0.11	2.04	1.14
0.1	0.31	1.83	1.28	1.85	0.72	1.08	1.39	1.26
0.3	1.29	1.85	1.2	0.84	1.30	2.08	2.50	1.03
1	0.71	1.93	1.23	0.19	0.82	2.56	1.20	1.35
3	0.06	0.74	0	0	1.03	1.36	0	0

APPENDIX B

Individual Subject Substitution Test and Response Rate Results in 1.0 mg/kg 4-MMC Training Group

Table 1

Individual Subject Performances for 4-MMC Substitution Tests and Response Rates in 1.0 mg/kg 4-MMC- trained Rats

Percent 4-MMC-Lever Selection								
	Subjects							
Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0	31.25	0	8.70	8.70	0	0	0	0
0.01	0	0	100	4.55	0	0	0	0
0.03	31.11	100	0	4.55	100	46.15	0	0
0.1	7.69	0	100	16	74.07	0	4	16
0.3	0	100	100	100	100	0	0	0
1	100	95.24	100	100	100	90.91	83.33	100
Response Rate								
Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0	1.67	3.33	1.38	1.77	2.20	2.15	2.56	0.36
0.01	1.96	3.77	1.69	1.58	2.13	2.06	2.53	1.22
0.03	1.76	3.39	0.98	1.51	1.63	2.20	2.27	0.89
0.1	1.68	2.90	1.28	2.38	2.29	1.55	2.17	1.60
0.3	1.80	2.78	1.69	2.63	1.64	1.44	2.47	1.72
1	1.92	2.23	1.67	1.94	1.90	1.35	1.03	2

Table 2

Individual Subject Performances for MDMA Substitution Tests and Response Rates in 1.0 mg/kg 4-MMC-trained Rats

Percent 4-MMC-Lever Selection								
	Subjects							
Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0	0	100	0	0	0	0	0	4.55
0.03	0	0	0	0	0	0	0	12.5
0.1	0	0	100	19.23	0	0	0	40
0.3	0	0	46.15	0	0	0	95.24	100
1	100	100	100	84.62	100	90.91	95.24	100
Response Rate								
Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0	2.22	0.62	1.05	1.71	2.08	2.30	1.69	1.72
0.03	1.89	4.00	1.00	2.86	1.92	1.48	3.02	1.52
0.1	1.98	2.86	1.38	1.26	2.53	2.44	2.78	1.67
0.3	1.79	4.65	0.66	2.22	1.64	2.60	1.50	1.41
1	1.64	1.36	1.27	1.52	1.44	0.79	2.00	1.92

Table 3

Individual Subject Performances for (+)-LSD Substitution Tests and Response Rates in 1.0 mg/kg 4-MMC-trained Rats

Percent 4-MMC-Lever Selection

	Subjects							
Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0.01	0	0	8.70	4.55	0	0	4.55	0
0.03	0	100	86.21	0	0	0	0	0
0.1	0	*DNC*	4.55	100	44.74	40.91	62.03	92.31
0.2	*DNC*	*DNC*	*DNC*	*DNC*	35.29	29.03	*DNC*	*DNC*

DNC = did not complete FR 20

Response Rate

Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0.01	2.08	5	0.84	2.37	2.56	2.33	1.46	1.09
0.03	2.11	1.96	1.05	2.11	1.64	1.41	1.68	1.48
0.1	0.57	0	0.85	0.80	0.61	0.22	0.09	0.05
0.2	0	0	0	0	0.08	0.07	0	0

Table 4

Individual Subject Performances for (+)-Fenfluramine Substitution Tests and Response Rates in 1.0 mg/kg 4-MMC-trained Rats

Percent 4-MMC-Lever Selection								
	Subjects							
Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0.1	0	100	12.5	41.67	*DNC*	0	0	28.81
0.3	0	0	0	0	0	41.67	46.67	100
1	0	23.4	88.89	4.55	100	95.24	100	46.15
3	25.49	*DNC*	*DNC*	100	96.88	48.53	97.56	100

DNC = did not complete FR 20

Response Rate								
Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0.1	2.78	0.75	1.36	1.55	0	2.13	2.67	1.09
0.3	2.33	0.68	1.33	1.01	0.43	0.91	0.75	0.89
1	0.94	0.47	0.66	1.36	0.75	1.14	0.96	0.73
3	0.16	0	0	0.36	0.09	1.05	1.02	0.07

Table 5

Individual Subject Performances for MDPV Substitution Tests and Response Rates in 1.0 mg/kg 4-MMC-trained Rats

Percent 4-MMC-Lever Selection								
	Subjects							
Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0.03	0	0	0	8.7	0	0	8.70	4.55
0.1	0	0	19.23	100	0	0	0	12.5
0.3	0	0	8.70	8.70	100	0	0	8.70
1	0	0	75	59.46	95.24	95.24	95.24	85.19
3	*DNC*	4.55	100	91.30	100	100	100	100
DNC = did not complete FR 20								
Response Rate								
Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0.03	1.12	3.28	0.99	0.96	2.20	2.41	1.78	1.38
0.1	2.25	2.02	0.64	1.67	1.61	2.67	1.98	1.24
0.3	1.47	1.16	1.16	1.72	1.47	1.69	2.17	1.36
1	0.17	0.19	1.46	1.32	0.50	0.94	0.06	0.9
3	0	0.10	1.02	2.56	0.33	1.59	0.28	1.28

Table 6

Individual Subject Performances for d-Amphetamine Substitution Tests and Response Rates in 1.0 mg/kg 4-MMC-trained Rats

Percent 4-MMC-Lever Selection								
	Subjects							
Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0	0	0	100	16	100	0	22.22	25
0.03	0	0	38.24	4.55	0	0	0	4.55
0.1	0	0	8	16	0	69.33	0	0
0.3	62.86	0	0	32.26	0	95.24	53.85	22.22
1	95.24	100	100	97.06	0	59.42	100	100
Response Rate								
Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0	1.23	4.17	1.56	1.13	2.82	2.08	2.27	1.56
0.03	2.35	4.83	1.05	1.48	1.69	2.67	1.79	1.64
0.1	1.83	4	0.91	1.55	2	1.23	1.6	1.25
0.3	0.60	4.55	0.70	0.84	3.85	0.89	1.76	0.6
1	0.39	0.34	0.98	0.34	1.61	1.06	1.37	1.69

Table 7

Individual Subject Performances for (+)-Methamphetamine Substitution Tests and Response Rates in 1.0 mg/kg 4-MMC-trained Rats

Percent MDPV-Lever Selection								
	Subjects							
Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0.03	0	0	4.55	4.55	0	0	0	4.55
0.1	100	0	90.91	100	100	0	0	100
0.3	5.41	58.82	100	94.29	0	8	0	100
1	95.24	100	100	100	0	0	86.96	100
3	*DNC*	*DNC*	*DNC*	*DNC*	*DNC*	100	100	100
DNC = did not complete FR 20								
Response Rate								
Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0.03	1.57	1.79	0.89	1.51	1.77	2.94	1.57	1.13
0.1	1.59	3.85	2.68	0.83	1.74	2.04	2.22	1.02
0.3	1.51	2.96	1.32	2.61	2.70	1.67	1.41	1.05
1	1.45	2.86	1.39	0.82	1.65	1.80	1.40	1.48
3	0	0	0	0	0	0.97	0.19	1.01

Table 8

Individual Subject Performances for (-)-Cocaine Substitution Tests and Response Rates in 1.0 mg/kg 4-MMC-trained Rats

Percent 4-MMC-Lever Selection								
	Subjects							
Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0	0	0	8.70	0	0	0	95.24	4.00
0.03	16.00	0	4.00	0	0	0	0	0
0.1	0	95.24	100	0	0	0	0	12.50
1	95.24	0	100	4.55	42.11	95.24	97.44	13.79
3	95.24	0	88.00	0	100	0	100	100
10	100	100	100	100	100	95.24	100	89.47
Response Rate								
Dose (mg/kg)	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**
0	1.79	3.70	0.69	0.82	2.62	2.13	1.27	1.79
0.03	1.21	2.20	0.91	0.65	1.98	1.54	2.30	0.93
0.1	1.92	2.63	1.03	1.14	2.08	2.44	2.35	1.58
1	1.98	4.65	1.54	1.96	1.77	1.15	2.13	1.41
3	0.49	1.23	2.02	1.90	0.86	2.35	2.11	1.18
10	1.82	0.41	1.74	1.03	0.12	1.64	2.02	1.07

APPENDIX C

IACUC Approval Form

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