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DIFFERENTIAL EFFECTS OF METHYLENEDIOXYPYROVALERONE (MDPV)
AND MEPHEDRONE IN RATS TRAINED TO DISCRIMINATE MDMA OR
A D-AMPHETAMINE+MDMA MIXTURE

by

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A thesis submitted to the Graduate College
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Eric Louis Harvey, M.A.

Western Michigan University, 2015

Recent reports on the abuse of novel synthetic cathinone derivatives call attention to serious public health risks of these substances. In response to this concern, a growing body of preclinical research has characterized the psychopharmacology of these substances, particularly mephedrone (MEPH) or methylenedioxypropylvalerone (MDPV), noting their similarities to MDMA and cocaine. The present study employed drug discrimination methodology to assess the discriminative stimulus effects of MEPH and MDPV in male Sprague-Dawley rats trained to discriminate 1.5 mg/kg 3,4-methylenedioxymethamphetamine (MDMA) or a mixture of 1.5 mg/kg MDMA and 0.5 mg/kg d-amphetamine (MDMA+AMPH). After establishing dose response functions with each training drug, MDPV (0.25-2.0 mg/kg), mephedrone (0.25-2.0 mg/kg), and cocaine were evaluated for substitution. Dose response curves generated with MDMA and MEPH were comparable between training groups. In contrast, AMPH, MDPV, and cocaine produced only partial substitution in animals trained to discriminate MDMA but produced full substitution in animals trained to discriminate the MDMA+AMPH mixture. These findings indicate MDPV's effects may be more similar to those of traditional psychostimulants, whereas MEPH exerts stimulus effects more similar to those of MDMA.

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Aut inveniam viam aut faciam

Eric Louis Harvey

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Introduction

Recent reports have noted a marked increase in the use of recreational “designer drugs” in the United States and Europe (Gibbons & Zloh, 2010; Rosenbaum et al., 2012). Of particular concern is the emergence and growing popularity of synthetic cathinone derivatives. Cathinone is the naturally occurring amphetamine-like alkaloid found in *Catha edulis* (Khat), a plant native to Africa and the Middle East. Although the extracts of Khat leaves have been used for centuries for their psychostimulant properties, medical and law enforcement reports of serious toxicities associated with synthetic cathinone derivatives have appeared only within the last decade in the U.S. (Goodnough & Zezima, 2011; Winstock & Ramsey, 2010).

The emergence of this public health threat began in the mid to late 2000s, when synthetic cathinones gained popularity among recreational drug users. Presumably as a method of diversion and evasion of FDA regulations, mixtures of synthetic cathinones were falsely marketed under a variety of product descriptions, such as “bath salts”, “plant food”, and “research chemicals”. Toxicities resulting from use of these products have received widespread media attention, including reports of violent and bizarre behavior (e.g. Campbell, 2012; "Police: Man on," 2013). In response to a growing public health concern, several of the chemical constituents of these products and their analogs are now classified as Schedule I controlled substances in the United States (DEA, 2011).

The chemical constituents of “bath salts” include several synthetic cathinone derivatives, presenting a considerable challenge to medical and scientific investigations

to determine which of these chemicals pose the greatest health threat. However, according to a recent report by the DEA, mephedrone (4-methylmethcathinone or 4-MMC), methylenedioxypropylvalerone (MDPV), and methylone (3,4-methylenedioxymethylcathinone) account for approximately 98% of all synthetic cathinones encountered by law enforcement agencies (DEA, 2011). There is now a growing body of literature devoted to preclinical investigations of the neuropharmacological and behavioral effects of synthetic cathinones. The majority of these studies have examined the effects of either mephedrone or MDPV, although a few have also included other cathinone derivatives (Wright et al., 2012; Baumann et al., 2012; Huang et al., 2012; Lisek et al., 2012; Motbey, 2012; Aarde et al., 2013; Varner et al., 2013; Shortall et al., 2013; Fantegrossi et al., 2013; Gatch et al., 2013). It is now well established that these substances dose-dependently increase locomotor activity in rodents. Furthermore, repeated daily dosing with mephedrone for five to seven days (Lisek et al., 2012; Gregg et al., 2013a; Berquist et al., 2015) as well as repeated intermittent dosing (Shortall et al., 2013) produced behavioral sensitization in rodents. Additionally, at least one study demonstrated cross-sensitization to the acute locomotor effects of cocaine (15 mg/kg) 10 days after a five day treatment regimen with mephedrone (15 mg/kg) in rats (Gregg et al., 2013b), although this effect was not bidirectional. Mephedrone (30 mg/kg) has also been shown to produce conditioned place preference (CPP) in both rats and mice (Lisek et al., 2012) and methylone was reported to produce CPP in mice (Miyazawa et

al., 2011). Moreover, mephedrone supports intravenous self-administration in rats (Aarde et al., 2013).

Drug discrimination methodology is commonly employed to characterize the neuropharmacological actions of novel psychoactive substances. Drugs sharing similar discriminative functions in nonhumans also tend to have common psychoactive effects (i.e. intoxicating effects) in humans (Young, 2009). Moreover, drugs that are determined to have similar discriminative stimulus properties can be predicted to share some pharmacological mechanisms of action as well as similar abuse liabilities (Nicholson & Balster, 2001). To date, four published studies have examined one or more of the synthetic cathinones using drug discrimination methodology with rodents. Methylone was reported to substitute fully in rats trained to discriminate d-amphetamine or MDMA from saline, but not in rats trained to discriminate DOM from saline (Dal Cason et al., 1997). More recently, Gatch et al. (2013) reported that several cathinone derivatives (MDPV, mephedrone, flephedrone, naphyrone, methylone, and butylone) all produced dose-dependent increases in drug-lever responding and fully substituted in male Sprague-Dawley rats trained to discriminate either cocaine (10 mg/kg) or methamphetamine (1 mg/kg). Varner et al. (2013) reported that male Long Evans hooded rats successfully acquired discrimination of 3.2 mg/kg mephedrone. Only MDMA and mephedrone produced complete substitution, while 18 mg/kg cocaine (76%) and 1 mg/kg methamphetamine (73%) produced partial substitution. Fantegrossi et al. (2013)

demonstrated full substitution with MDMA and methamphetamine in male NIH Swiss mice trained to discriminate 0.3 mg/kg MDPV from saline.

In consideration of the fact that most psychoactive have complex stimulus functions involving several pharmacological mechanisms, drug discrimination procedures have been utilized by some researchers to evaluate the effects of drug mixtures in comparison to novel substances as a way to assess distinct components of a drug's complex stimulus functions (Stolerman, 2011). The goal of the present study was to compare stimulus generalization with the synthetic cathinones, MDPV and mephedrone, in rats trained to discriminate either a drug mixture (d-amphetamine + MDMA) or a single drug (MDMA).

Methods

Subjects

Sixteen adult male Sprague-Dawley rats were housed individually in polycarbonate cages lined with corn cob bedding (Harlan Teklad, Conrad, Iowa) in animal facilities maintained at constant temperature ($20\pm 2^{\circ}\text{C}$) and humidity ($50\pm 5\%$) under a 12:12 light/dark cycle, (lights on from 0900 to 2100). Water was provided ad libitum in the home cages. Commercial rodent diet (Purina® 5001, Richmond, Indiana) was restricted to daily feeding to maintain animals at 80-90% of free-feeding weights. All procedures were reviewed and approved by the Western Michigan University Institutional Animal Care and Use Committee and were in accordance with the guidelines

of the *Guide for the Care and Use of Laboratory Animals* (National Academy of Sciences, 2011) and EU Directive 2010/63/EU.

Apparatus: Training and testing were conducted in eight sound-attenuated operant conditioning chambers (ENV-001; MED Associates Inc., Georgia, VT, USA) equipped with three removable levers and a food pellet dispenser located on the front panel, a 28-V house light, and fan. Reinforcers for lever pressing consisted of 45 mg Dustless Precision Pellets® (Product# F0021, BioServ, Flemington, NJ). Experimental events were programmed and controlled using Med-PC software (version IV; MED Associates Inc., St. Albans, VT, USA).

Drugs

Mephedrone-hydrochloride, 3,4-methylenedioxypropylamphetamine-hydrochloride, cocaine-hydrochloride, and 3,4-methylenedioxymethamphetamine-hydrochloride were generously provided by the National Institute on Drug Abuse drug control supply program (Bethesda, MD). d-Amphetamine-hemisulfate was purchased from Sigma Chemical Company (St Louis, MO). All drugs were dissolved in bacteriostatic 0.9% sodium chloride with a constant injection volume of 1ml/kg administered by intraperitoneal (i.p.) injection. For the training drug mixture of d-amphetamine and MDMA, these substances were dissolved together in a single solution. Doses were calculated based on the weights of the salts.

Preliminary Training

Subjects were acclimated to the operant chambers for two 60 minute sessions, one per day for two consecutive days. During these acclimation sessions, no levers were extended and food pellets were delivered under a fixed-time 60 sec (FT60") schedule to familiarize the animals with the location of the food magazine and sound of the pellet dispenser. Subsequent training sessions lasted 20 min per day and were conducted five to six days per week. Animals were initially trained to lever press with only the center lever extended and reinforcement was delivered under a fixed-ratio (FR) schedule that was gradually incremented from FR 1 to FR 20 over the course of seven training sessions. Once subjects were reliably lever pressing on the FR 20 schedule, errorless training sessions were conducted with either the left lever or right lever extended. During this phase, subjects received injections of either the training drug (see below) or saline 10 min prior to the beginning of each session. Half the animals in each training group were reinforced for responses on the right lever following drug injections and for responses on the left lever following saline injections. Conditions were reversed for the remaining animals in each group. A total of 12 errorless training sessions were conducted in the following order: V, V, D, D, V, D, V, V, D, D, V, D. Once subjects were responding reliably on an FR20 schedule for both the drug-paired and vehicle-paired levers, discrimination training commenced.

Discrimination Training

Both the left and right levers were present during discrimination training sessions. These sessions were 20 min in duration and were conducted only once per day, five to six days a week. One group of rats (n=7) was trained to discriminate 1.5 mg/kg MDMA from saline injections and the other group (n=8) was trained to discriminate a mixture of 1.5 mg/kg MDMA + 0.5 mg/kg d-amphetamine from saline injections. Similar to the preliminary training sessions, responding was initially reinforced under a FR1 schedule that was progressively incremented to a FR20 schedule under drug and vehicle conditions, independently based on each subject's performance. Once animals were reliably responding under the FR20 schedule under both drug and vehicle conditions, this schedule remained in effect for the remainder of the training sessions. Drug and vehicle training sessions were alternated with sessions under the same stimulus conditions occurring no more than twice consecutively. The performance criteria for stimulus control was a minimum of 8 out of 10 consecutive discrimination trials with an 80% or better correct lever response prior to delivery of the first reinforcer and for the total session.

Stimulus Generalization Tests

When the discrimination criteria were met, stimulus generalization tests commenced and dose-response curves were established with the following test compounds: MDMA (0.19 – 1.5 mg/kg), AMPH (0.25 – 2.0 mg/kg), cocaine (1.25 – 10 mg/kg), (+)-MDMA (0.375 – 3.0 mg/kg), mephedrone (0.25 – 2.0 mg/kg), and MDPV

(0.13 – 3.0 mg/kg). All compounds were administered 10 min prior to each test session. Test sessions were conducted under extinction and ended immediately following the completion of 20 consecutive responses on either lever or when 20 min elapsed, whichever occurred first. The order of the test doses were counterbalanced among animals in each training group. Approximately half of the animals in each group were tested with a particular dose following a drug training session, and the other half was tested following a vehicle training session. Each subject completed a minimum of one drug and one vehicle training session between generalization test sessions and was required to meet the 80% discrimination criteria on the most recent drug and vehicle training sessions prior to each test.

Data Analysis

The mean (\pm SEM) number of sessions to criterion was calculated for each training group and statistically analyzed with a t-test. Dose-response curves were graphed for each training drug and test compound, with the mean (\pm SEM) percentage of drug-appropriate lever responses as well as the mean (\pm SEM) response rate (lever presses per second) plotted as a function of dose. Data from subjects completing test sessions with all doses of a compound were statistically evaluated using a mixed model two-way analysis of variance (ANOVA) with training drug as a between subjects comparison and test dose as a within subjects comparison. For drugs that produced full substitution (80% or higher drug-lever responding at any dose), a nonlinear regression was conducted on the dose-

response curve to estimate ED₅₀ values. Statistical analyses were conducted and graphs were created using GraphPad Prism (version 6.0) software (La Jolla, CA, USA).

Results

Discrimination Acquisition

Rats trained to discriminate MDMA+AMPH met the specified criteria for discrimination within 16.9 (± 0.4 , SEM) training sessions (range 16 – 19) while rats trained to discriminate MDMA met these criteria in an average of 29.1 (± 4.4 , SEM) training sessions (range 16 – 43). This difference was statistically significant ($t(6.1) = 2.781, p < 0.05$). Interestingly, there was a bimodal distribution in the sessions to criteria among the MDMA training group, with a range of 16 to 18 sessions among three animals and a range of 35 to 43 sessions among the other four animals.

Stimulus Generalization

Dose response curves for MDMA, AMPH, and cocaine are displayed in Figure 1. Dose response curves for (+)-MDMA, mephedrone, and MDPV are displayed in Figure 2. MDMA produced a dose-dependent increase in drug-appropriate responding and substituted fully at 1.5 mg/kg in both MDMA-trained and MDMA+AMPH-training groups. A two-factor mixed model ANOVA showed a statistically significant effect of MDMA dose on the percentage of drug-lever responding ($F_{4, 52} = 23.96, p = < 0.001$), although there was no significant effect of training group, nor was there a significant training group by test dose interaction. Bonferroni multiple comparison tests indicated

that the 0.75 and 1.5 mg/kg doses in both groups, as well as the 0.19 mg/kg dose in the MDMA training group, were significantly different from saline ($p < 0.05$).

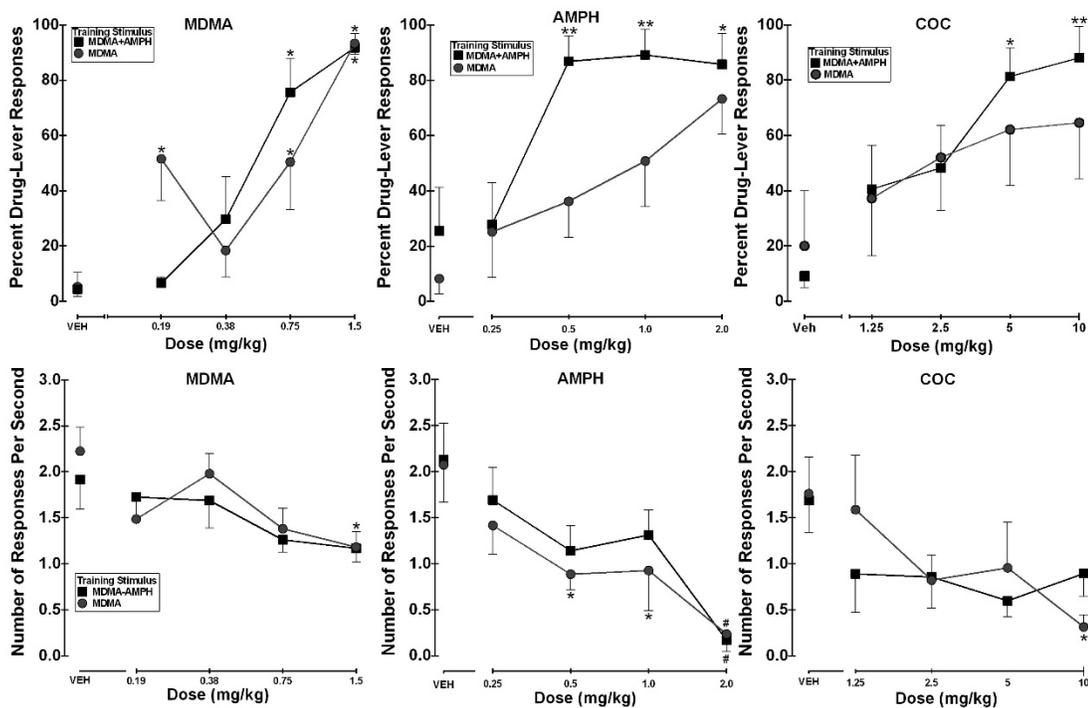


Figure 1. Dose response curves determined from stimulus generalization tests with MDMA, d-amphetamine, and cocaine in rats trained to discriminate a 1.5 mg/kg MDMA ($n = 7$) or a mixture of 1.5 mg/kg MDMA + 0.5 mg/kg amphetamine ($n = 8$) from saline. Graphs in the upper panel depict percentage of responses on the drug-appropriate lever. Graphs in the lower panel depict response rate. Individual points represent group means (\pm S.E.M.). MDMA+AMPH mixture group (■) and MDMA alone group (●). Significant Bonferroni multiple comparison tests between selected doses and saline are represented by * ($p < 0.05$), ** ($p < 0.01$), and # ($p < 0.001$).

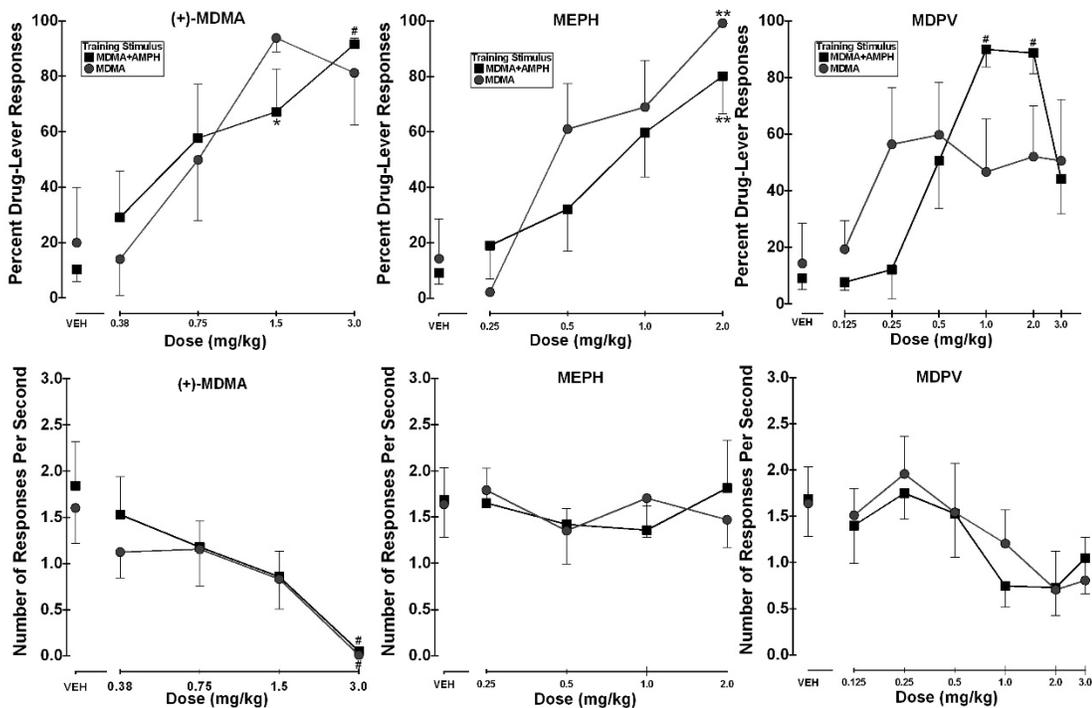


Figure 2. Dose response curves determined from stimulus generalization tests with (+)-MDMA, mephedrone, and MDPV in rats trained to discriminate a 1.5 mg/kg MDMA ($n = 7$) or a mixture of 1.5 mg/kg MDMA + 0.5 mg/kg amphetamine ($n = 8$) from saline. Graphs in the upper panel depict percentage of responses on the drug-appropriate lever. Graphs in the lower panel depict response rate. Individual points represent group means (\pm S.E. M.). MDMA+AMPH mixture group (■) and MDMA alone group (●). Significant Bonferroni multiple comparison tests between selected doses and saline are represented by * ($p < 0.05$), ** ($p < 0.01$), and # ($p < 0.001$).

The ED_{50} values for MDMA were 0.21 mg/kg (95% CI [0.12 – 0.37 mg/kg]) and 0.35 mg/kg (95% CI [0.15 – 0.82 mg/kg]) in the MDMA+AMPH and the MDMA training groups respectively. A two-factor mixed model ANOVA also showed a statistically significant main effect of test dose on response rate ($F_{4, 52} = 5.56, p < 0.001$). There was no statistically significant effect of training group nor was there a significant training

group by test dose interaction on response rate. Bonferroni multiple comparison tests indicated 1.5 mg/kg was significantly different from saline ($p < 0.05$) only in the MDMA-trained animals.

d-Amphetamine (AMPH) also produced a dose-dependent increase in drug-appropriate responding in both training groups. However, full substitution was observed in the MDMA+AMPH training group at 0.5, 1.0 and 2.0 mg/kg, whereas only partial substitution was observed in the MDMA training group. A two-factor mixed model ANOVA excluded data from two animals in the MDMA group and one animal in the MDMA+AMPH group that failed to respond following the 2.0 mg/kg dose. AMPH produced a statistically significant effect on the percentage of drug-lever responding ($F_{4, 40} = 7.07, p < 0.001$). A significant difference between training groups on drug-lever responding was also found ($F_{1, 10} = 11.34, p < 0.01$), but there was no significant training group by test dose interaction. Bonferroni multiple comparison tests revealed 0.25, 0.5 ($p < 0.01$) and 1.0 mg/kg AMPH ($p < 0.05$) produced drug-appropriate responding that was significantly different from saline in the MDMA+AMPH training group and none of the multiple comparisons were statistically significant in the MDMA training group. The ED_{50} for AMPH in the MDMA+AMPH training group was calculated at 0.06 mg/kg (95% CI [0.03 – 0.14 mg/kg]). A two-factor mixed model ANOVA found that response rate was significantly affected by test dose ($F_{4, 52} = 15.91, p < 0.001$). No significant effects of training group or test dose by training group were found. Bonferroni multiple comparison tests found the 2.0 mg/kg dose to be significantly different from saline in the

MDMA+AMPH training group ($p < 0.001$), and all but the 0.25 mg/kg dose to be significantly different from saline in the MDMA training group (0.5 and 1.0 mg/kg, $p < 0.05$ and 2.0 mg/kg, $p < 0.001$).

Similar to AMPH, cocaine also produced only partial substitution in animals trained to discriminate MDMA, but fully substituted in those trained to discriminate the MDMA+AMPH mixture. A mixed model two factor ANOVA showed a statistically significant main effect of cocaine dose on drug-lever responding ($F_{4, 40} = 4.428$, $p < 0.01$), although no significant effect of training group was observed, nor was there a significant training group by test dose interaction. Bonferroni multiple comparisons found that drug-appropriate responses were significantly different from saline in the MDMA+AMPH training group at the 5.0 mg/kg ($p < 0.05$) and 10.0 mg/kg ($p < 0.01$) doses. The ED_{50} value for animals trained to discriminate MDMA+AMPH was 4.65 mg/kg (95% CI [1.31 – 16.57 mg/kg]). A two-factor mixed model ANOVA also found a significant main effect of test dose on response rates ($F_{4, 40} = 3.70$, $p < 0.05$). No significant effects of training group or training group by test dose interaction were found. Bonferroni multiple comparison tests found response rate at the 10.0 mg/kg dose to be significantly lower than that of saline in the MDMA-trained group ($p < 0.05$).

The (+)-MDMA isomer produced a dose-dependent increase in drug-lever responding, with full substitution in the MDMA training group at the 1.5 mg/kg dose, and at the 3.0 mg/kg dose in both training groups. A two-factor mixed model ANOVA excluded data from five animals in the MDMA group and one animal in the

MDMA+AMPH group that did not complete the 3.0 mg/kg dose and indicated a significant main effect of (+)-MDMA dose on drug-lever responding ($F_{4, 28} = 8.245, p < 0.001$). No significant effects of training group or training group by test dose interaction were shown. Bonferroni multiple comparisons found that drug-lever responding at the 1.5 mg/kg and 3.0 mg/kg doses was significantly different from vehicle in both MDMA+AMPH group ($p < 0.05$ and $p < 0.001$, respectively). The ED_{50} values for (+)-MDMA were calculated at 0.44 mg/kg (95% CI [0.17 to 1.12 mg/kg]) in the MDMA+AMPH training group and 0.48 mg/kg (95% CI [0.19 to 1.19 mg/kg]) in the MDMA training group. A two-factor mixed model ANOVA also showed a significant main effect of (+)-MDMA dose on response rate ($F_{4, 32} = 11.64, p < 0.001$). Bonferroni multiple comparison tests revealed response rate at the 3.0 mg/kg to be significantly different from saline in both training groups ($p < 0.001$).

Mephedrone produced a dose-dependent increase in drug-appropriate responding and full substitution at the 2.0 mg/kg dose in both training groups. A two-factor mixed model ANOVA found a significant main effect of MEPH dose on drug-lever responding ($F_{4, 48} = 11.21, p < 0.001$). No significant effects of training group or training group by test dose interaction were shown. Bonferroni multiple comparisons revealed that drug-lever responding at the 2.0 mg/kg dose was significantly different from saline in both training groups ($p < 0.01$). The ED_{50} values were calculated at 0.56 mg/kg (95% CI [0.25 – 1.23 mg/kg]) in the MDMA+AMPH training group and 0.22 mg/kg (95% CI [0.10 –

0.49 mg/kg)) in the MDMA training group. A two-factor mixed model ANOVA revealed no significant effects of MEPH on response-rate.

Dose response curves for MDPV were distinctly different in the two training groups, similar to the distinction evident with cocaine and AMPH. As such, full substitution with MDPV was attained only in the MDMA+AMPH training group. A two-factor mixed model ANOVA found that the MDPV test dose had a significant effect on percent drug-lever responding ($F_{6,66} = 7.77, p < 0.001$). A significant test dose by training group interaction was also found ($F_{6,66} = 3, p < 0.05$), but there was no statistically significant effect of training group alone. Bonferroni multiple comparison tests showed that the 1.0 and 2.0 mg/kg doses were significantly different from saline in the MDMA+AMPH training group ($p < 0.001$). The ED_{50} value for MDPV in the MDMA+AMPH training group was calculated at 0.30 mg/kg (95% CI [0.11 – 0.82 mg/kg]). A two-factor mixed model ANOVA also showed a significant main effect of MDPV dose on response rate ($F_{5,65} = 3.460, p < 0.01$). Bonferroni multiple comparison tests did not reveal any individual doses to be significantly different from saline in either training group.

Discussion

As designer drugs continue to increase in popularity, due mostly to the ubiquity of internet sources from which to obtain them, the potential adverse psychological effects of these drugs are a growing public health concern. At the forefront of these new drugs are

the many variations of the synthetic cathinones, with mephedrone and MDPV among the most widely abused constituents and often found together in “bath salt” mixtures.

The primary finding of the present study is the differential substitution produced by mephedrone and MDPV in animals trained to discriminate MDMA or a complex drug mixture consisting of MDMA and d-amphetamine. Specifically, mephedrone produced similar dose-dependent increases in responding and reached full substitution in both training groups at the 2.0 mg/kg dose. Although not statistically significant, the ED₅₀ of mephedrone was approximately two-fold lower in the MDMA training group compared to the MDMA+AMPH training group. In contrast, MDPV produced full substitution only in the MDMA+AMPH training group, whereas a flat dose response curve and partial substitution was obtained with MDPV in the MDMA only training group. It is possible that higher MDPV doses could produce substitution for MDMA, although the rate suppressant effects precluded testing higher doses of MDPV. In fact, 3.0 mg/kg produced less substitution than 2.0 mg/kg in MDMA-trained animals, producing an inverted U shaped dose response function.

The full substitution of mephedrone in both training groups suggests that this substance produces similar interoceptive stimuli (i.e., subjective effects) to those produced by MDMA (the component common to both training groups). This hypothesis is consistent with self-reports by human subjects equating the subjective effects of mephedrone to those of MDMA (Carhart-Harris et al., 2011). Moreover, recent reports indicate the pharmacological mechanisms of action of mephedrone closely resemble

those of MDMA and are distinct from those of MDPV (Cameron et al., 2013; Bauman et al., 2012; Kehr et al., 2011). For example, unlike MDPV, mephedrone produces significant increases in serotonin (5-HT) release in rat nucleus accumbens *in vivo* (Kehr et al. 2011; Baumann et al. 2012), whereas MDPV reported blocks dopamine reuptake, similar to cocaine (Cameron et al., 2013).

Full substitution of MDPV only in the MDMA+AMPH training group, indicates that MDPV produces interoceptive stimuli that are dissimilar to those produced by MDMA alone and more similar to the amphetamine component of the MDMA+AMPH mixture. This is supported by the current results that both AMPH and cocaine also produced full substitution in the MDMA+AMPH training group, suggesting that amphetamine was the dominant component of the complex stimulus cue.

Due to the apparent lack of overshadowing by either component in the MDMA+AMPH mixture (i.e. both components of the mixture fully substituted individually at their respective training doses), it can be concluded that the MDMA+AMPH mixture does not produce a novel stimulus cue, but rather the components likely have an additive effect. These findings may be compared to those of Shoaib et al. (1997) who found that a mixture of fenfluramine (FEN) and phentermine (PHEN) (agents with similar neurochemical effects to MDMA and amphetamine, respectively) produced an additive cue in animals trained to discriminate a FEN+PHEN mixture from saline.

Insofar as drug discrimination offers a model of subjective drug effects, the current results are relevant to distinguishing the subjective effects of mephedrone and MDPV and the pharmacological actions contributing to these effects. Utilizing drug mixtures as complex stimuli offers a novel approach to examine the pharmacological mechanisms that may distinguish the subjective effects of mephedrone and MDPV. This study represents the first attempt to do so. In consideration of the common practice of polysubstance use, further investigations on the stimulus functions of drug mixtures may help elucidate the unique subjective effects of commonly co-abused drugs.

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Appendix

IACUC Protocol Approval Letter

WESTERN MICHIGAN UNIVERSITY



Institutional Animal Care and Use Committee

Date: March 13, 2013

To: Lisa Baker, Principal Investigator

From: Robert Eversole, Chair

Re: IACUC Protocol Number 13-03-03

A handwritten signature in black ink, appearing to be "RE", is written over the "From:" line.

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: March 13, 2014

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