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Intravenous self-administration of mephedrone, methylone and MDMA in female rats

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Abstract

Male rats will intravenously self-administer (IVSA) the substituted cathinone stimulants ("bath salts") mephedrone (4-methylmethcathione) and methylone (3,4-methylenedioxymethcathinone) robustly, whereas the IVSA of 3,4-methylenedioxymethamphetamine (MDMA) is inconsistent in many rat models. There are no data available on the self-administration of these drugs in female rats, thus a study was undertaken to contrast them directly. Groups of female Wistar rats were trained to self-administer mephedrone, methylone or MDMA (0.5 mg/kg/inf) under a Fixed-Ratio (FR) 1 schedule of reinforcement for 14 sessions. Following the acquisition interval, animals were evaluated in FR (0.0, 0.125, 0.25, 0.5, 1.0, 2.5 mg/kg/inf) and PR (0.125, 1.0 mg/kg/inf) dose-substitution procedures. The results show that female rats acquired the self-administration of all three compounds with intakes in mephedrone-trained rats that were significantly higher than that of methylone-trained or MDMA-trained rats. In dose-substitution under either FR or PR contingencies, however, the potencies of all three drugs were similar within the original training groups. The mephedrone-trained animals exhibited higher intakes of all drugs during dose-substitution, indicating lasting consequences of the training drug. Abuse liability of these three compounds is therefore predicted to be similar in established stimulant users but may differ in liability if they are primary drugs of initiation.

Keywords

Drug addiction; bath salts; Ecstasy; reward; substance abuse

1. INTRODUCTION

The emergence of two cathinone-class stimulant drugs mephedrone (4-methylmethcathinone; 4MMC) and methylone (3,4-methylenedioxymethcathinone), that each produce profiles of relatively enhanced accumulation of serotonin versus dopamine in

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the nucleus accumbens (Baumann et al., 2012; Kehr et al., 2011; Wright et al., 2012) similar to 3,4-methylenedioxymethamphetamine (MDMA) provide a key opportunity to further determine abuse liability of designer stimulant drugs. The intravenous self-administration (IVSA) of 3,4-methylenedioxymethamphetamine (MDMA) is not robust in rat models (De La Garza et al., 2007; Feduccia et al., 2010), is often marked by inter-subject inconsistency (Dalley et al., 2007) and in some paradigms approximately 40–50% of the sample fails to meet specific acquisition criteria (Colussi-Mas et al., 2010; Oakly et al., 2014; Schenk et al., 2007). Given correlations of relative dopamine/serotonin activity with reinforcer efficacy (Roberts et al., 1999; Wee et al., 2005) it has been assumed that the preferential enhancement of serotonin versus dopamine accumulation in the nucleus accumbens produced by MDMA (Baumann et al., 2008) explains its reduced reinforcer efficacy in rodent IVSA compared with the more classical amphetamines. A limited set of laboratory investigations shows that the substituted cathinone stimulants (“bath salts”) mephedrone and methylone are readily self-administered by male rats (Aarde et al., 2013a; Hadlock et al., 2011; Motbey et al., 2013; Watterson et al., 2012). A more direct comparison of these compounds is needed since there are significant methodological differences across these early studies.

It has long been established that significant sex-differences exist in rat models of stimulant drug abuse. For example, female rats will self-administer more cocaine (Roth and Carroll, 2004b; Smith et al., 2011) and more methamphetamine (Reichel et al., 2012; Roth and Carroll, 2004a) than males and these sex differences can be more pronounced under long-access escalation and/or Progressive Ratio procedures. It is currently unknown, in contrast to the classical stimulants, if female rats will also exhibit this enhanced self-administration of *atypical* stimulants such as MDMA or the recently emerging synthetic cathinones mephedrone and methylone. Thus, the present study was conducted in female rats to expand understanding of the comparative reinforcing properties of these compounds that have similar neuropharmacological effects (Baumann et al., 2012; Kehr et al., 2011; Wright et al., 2012). This choice also provides the opportunity to determine if prior results with MDMA self-administration generalize to female rats.

This study was undertaken to directly compare the likely abuse liability of mephedrone, methylone and MDMA using the rat intravenous self-administration model of drug reinforcement. The primary focus was to contrast the potency and efficacy of each drug using a Fixed Ratio and Progressive Ratio procedures with dose-substitution. All three drugs were examined in each training group in a design consistent with recent recommendations for comparing stimulant drug efficacy (Huskinson et al., 2014).

2.METHODS

2.1 Subjects

Female Wistar rats (Charles River, New York) were used for these investigations. Animals were housed in a humidity and temperature-controlled (23±1 °C) vivarium on 12:12 hour light:dark cycles. Animals entered the laboratory at 11–12 weeks of age and weighed 174–266 grams at the start of the self-administration study. Animals had *ad libitum* access to food and water in their home cages. All procedures were conducted in the dark cycle, under

protocols approved by the Institutional Care and Use Committees of The Scripps Research Institute and consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2 Intravenous catheterization

Rats (mephedrone, N = 18; methylone, N=18; MDMA, N = 18) were anesthetized with an isoflurane/oxygen vapor mixture (isoflurane 5% induction, 1–3% maintenance) and prepared with chronic intravenous catheters as described previously (Aarde et al., 2013a; Aarde et al., 2013b; Aarde et al., 2014). Briefly, the catheters consisted of an 14-cm length of polyurethane based tubing (Micro-Renathane®, Braintree Scientific, Inc, Braintree MA, USA) fitted to a guide cannula (Plastics One, Roanoke, VA) curved at an angle and encased in dental cement anchored to an ~3 cm circle of durable mesh. Catheter tubing was passed subcutaneously from the animal's back to the right jugular vein. Catheter tubing was inserted into the vein and tied gently with suture thread. A liquid tissue adhesive was used to close the incisions (3M™ Vetbond™ Tissue Adhesive; 1469SB).

A minimum of 4 days was allowed for surgical recovery prior to starting an experiment. For the first three days of the recovery period, an antibiotic (cephazolin) and an analgesic (flunixin) were administered daily. During testing and training, intravenous catheters were flushed with heparinized saline before sessions and heparinized saline containing cefazolan (100 mg/mL) after sessions.

Catheter patency was assessed nearly once a week after the last session of the week via administration through the catheter of ~0.2 ml (10 mg/ml) of the ultra-short-acting barbiturate anesthetic Brevital sodium (1% methohexital sodium; Eli Lilly, Indianapolis, IN). Animals with patent catheters exhibit prominent signs of anesthesia (pronounced loss of muscle tone) within 3 sec after infusion. Animals that failed to display these signs were considered to have faulty catheters and were discontinued from the study. Data that was taken prior to failing this test and after the previous passing of this test were excluded from analysis.

2.3 Drugs

The racemic 4-methylmethcathinone (mephedrone) HCl used for this study was obtained from Fox Chase Chemical Diversity Center (Doylestown, PA) from routes designed by Tobin J. Dickerson, Ph.D.. Racemic 3,4-methylenedioxymethamphetamine (MDMA) HCl was provided by the National Institute on Drug Abuse’s Drug Supply Program. Racemic 3,4-methylenedioxymethcathinone (methylone) HCl was obtained from Cayman Chemical. All doses are expressed as the salt and were dissolved in physiological saline for injection.

2.4 Self-administration procedure

Behavioral studies were conducted in three phases consisting of a 14 session Acquisition phase, a 4-week Fixed-Ratio dose-substitution phase and a 6 session Progressive-Ratio dose-substitution phase, as described below and outlined in Figure 1.

2.4.1 Acquisition—Drug self-administration was conducted in operant boxes (Med Associates) located inside sound-attenuating chambers located in an experimental room (ambient temperature 23 ± 1 °C; illuminated by red light) outside of the housing vivarium. To begin a session, the catheter fittings on the animals' backs were connected to polyethylene tubing contained inside a protective spring suspended into the operant chamber from a liquid swivel attached to a balance arm. Each two hour operant session started with the extension of two retractable levers into the chamber. Following each completion of the response requirement (response ratio), a white stimulus light (located above the reinforced lever) signaled delivery of the reinforcer and remained on during a 20-sec post-infusion timeout, during which responses were recorded but had no scheduled consequences. Drug infusions were delivered via syringe pump. The acquisition interval of 14 sessions was selected *a priori* from prior studies, particularly those involving MDMA and methylone self-administration (Dalley et al., 2007; Schenk et al., 2007; Watterson et al., 2012), since prior mephedrone studies involved food-reinforced lever-training (Aarde et al., 2013a; Hadlock et al., 2011).The training dose for all three drugs (0.5 mg/kg/infusion; ~0.1 mL / infusion) was selected from prior self-administration studies (Aarde et al., 2013a; Hadlock et al., 2011; Watterson et al., 2012) as well as comparison of mephedrone vs MDMA potency in locomotor and thermoregulatory studies (Aarde et al., 2013a; Huang et al., 2012; Miller et al., 2013; Wright et al., 2012).

2.4.2 Fixed- Ratio (FR) Dose-Response Testing—The treatment sequence for all three groups is outlined in Figure 1. The mephedrone-trained and MDMA-trained groups were initially subjected to randomized dose-substitution conditions in which the per-infusion dose of their respective training drug differed (0.0, 0.25, 0.5, 1.0, 2.5 mg/kg/inf) on sequential two hour sessions immediately following acquisition. These two groups were next evaluated with sequential substitution of doses of methylone (0.0, 0.25, 0.5, 1.0, 2.5 mg/kg/inf). Next, the group originally trained on mephedrone was evaluated on substituted doses of MDMA (0.0, 0.125, 0.25, 0.5, 2.5 mg/kg/inf) and the group originally trained on MDMA on doses of mephedrone (0.0, 0.125, 0.25, 0.5, 2.5 mg/kg/inf). These two groups ended FR evaluation with a repeat of their original training drug (0.0, 0.125, 0.25, 0.5, 2.5 mg/kg/inf in both cases). For the methylone-trained group, the FR studies were methylone (0.0, 0.125, 0.25, 0.5, 2.5 mg/kg/inf), mephedrone (0.0, 0.125, 0.25, 0.5, 2.5 mg/kg/inf), MDMA (0.0, 0.125, 0.25, 0.5, 2.5 mg/kg/inf) and a repeat of methylone (0.0, 0.125, 0.25, 0.5, 2.5 mg/kg/inf). The dose order within each drug series was balanced by Latin Square design.

2.4.3 Progressive- Ratio (FR) Dose-Response Testing—Following the FR evaluation, the animals in all three groups were subjected to randomized dose-substitution conditions in which different per-infusion doses (0.125, 1.0 mg/kg/inf) of MDMA, methylone or mephedrone were presented on sequential sessions. In this self-administration paradigm, the required response ratio increases after each reinforcer delivery (Hodos, 1961; Segal and Mandell, 1974) as determined by the following equation (rounded to the nearest integer): Response Ratio = 5e^(injection number * j) – 5 (Richardson and Roberts, 1996). The value of “j” for this study was 0.3 and was based upon a prior study with methamphetamine (Aarde et al., 2013b) so as to observe a “breakpoint” in ~3 hrs. The drug

and dose order was balanced by Latin Square design (i.e., the 6 total conditions were randomized). The last ratio completed before the end of the session was operationally defined as the breakpoint.

2.5 Data Analysis

Due to prior observations of significant individual differences in the acquisition of MDMA IVSA (Colussi-Mas et al., 2010; Oakly et al., 2014; Schenk et al., 2007), the primary analysis of the acquisition data included the discrimination ratio (drug associated lever / all lever responses) as a dependent variable and a secondary median split analysis was conducted for the initial acquisition data. The data were analyzed with repeated-measures Analysis of Variance (rmANOVA) with dose and session number as within-subjects factors and drug identity and intake preference (median split) as between-subjects factors as appropriate. Any significant rmANOVA main effects were followed with post-hoc analysis using Tukey correction for all possible comparisons. Analyses were conducted using Prism 6 for Windows (v. 6.02; GraphPad Software, Inc, San Diego CA) or StatView (SAS Institute, Inc, Cary, NC). Graphs were generated with Excel (Microsoft, Redmond WA) and figures created in Canvas(v.12; ACD Systems of America, Inc, Seattle, WA)

3. RESULTS

3.1 ACQUISITION

3.1.1 Infusions—Groups of female rats acquired self-administration while being trained on 0.5 mg/kg/inf of MDMA (N=15), methylone (N=16) and Mephedrone (N=16) as is shown in Figure 2. The ANOVA confirmed significant effects of training Session [$F(13, 572) = 16.50$; $P < 0.0001$], of training Drug [$F(2, 44) = 6.344$; $P < 0.0001$] and the interaction [$F(26, 572) = 3.361$; $P < 0.0001$] for infusions obtained. The posthoc test (Tukey) confirmed mephedrone group obtained more infusions than the methylone (Sessions 4–5, 8–14) or MDMA (Sessions 7–14). Intake of the methylone and MDMA groups did not differ at any session. Likewise the post-hoc test confirmed significantly more mephedrone infusions were obtained relative to Session 1 (Sessions 5–14), Session 2 (Sessions 4–5, 7–14), Session 3 (Sessions 8–14), Session 4,5 and 7 (Sessions 13–14), Session 6 (Sessions 10, 12–14) and Session 8 (Session 14). Significantly more methylone was obtained in Session 14 compared with Sessions 1–7 and 9 and in Session 13 relative to Sessions 1 and Sessions 3–4. No differences in MDMA intake across sessions were confirmed by the posthoc test.

3.1.2 Drug-Lever Discrimination—A second rmANOVA analysis confirmed that the drug-associated lever discrimination ratio (Figure 3) was significantly affected by training Session [$F(13, 572) = 9.82$; $P < 0.0001$], but not by training Drug [$F(2, 44) = 1.78$; $P = 0.18$] or the interaction [$F(26, 572) = 1.06$; $P = 0.38$]. The post hoc test confirmed that drug-lever discrimination in the Mephedrone-trained group was significantly improved over Session 1 and 2 in Sessions 9,11,13–14, over Sessions 3 and 4 in Sessions 8–14 and over Session 3 compared in Session 6. The post hoc test also confirmed that lever discrimination in the MDMA-trained animals was lower in Session 1 compared with sessions 11 and 14. The post hoc test did not confirm any difference in lever discrimination within the Methylone group across the acquisition interval.

3.2 MEDIAN SPLIT

3.2.1 Infusions—The acquisition groups were divided in half by average daily infusions obtained across the initial 14 sessions (the odd animal in the MDMA group was assigned to the upper group) resulting in a two-way mixed analysis with six treatment groups (3 drugs by 2 distribution halves) and 14 repeated IVSA sessions (Figure 2, **middle and lower panels**). The ANOVA for infusions confirmed significant effects of training Session [$F(13, 533) = 18.82$; $P < 0.0001$], of Group [$F(5, 41) = 27.39$; $P < 0.0001$] and the interaction [$F(65, 533) = 3.52$; $P < 0.0001$]. The post-hoc test first confirmed that there were significant more infusions obtained by the Mephedrone-upper group compared with each of the three lower half groups from session 4–14. The Mephedrone-upper group also obtained significantly more infusions than the Methylone-upper group (sessions 4, 9–14) and MDMA-upper group (Sessions 7–14). The posthoc test likewise confirmed that Methylone-upper animals obtained more infusions than the Methylone-lower (Sessions 8–14), MDMA-upper (Session 14), Mephedrone-lower (Session 14) and MDMA-lower (Sessions 8, 10–14) groups. Significantly more infusions were obtained by the MDMA-upper group compared with the MDMA-lower (Session 13–14) and Methylone-lower (Session 13) groups. Finally, the Mephedrone-lower group obtained more infusions than the MDMA-lower group on Session 14.

Within the median split drug groups, the post-hoc test confirmed significant increases in the Mephedrone-upper group over time since more infusions were obtained in Sessions 4–14 compared with sessions 1 or 2, more in Sessions 8–14 compared with Session 3, more in sessions 10–14 compared with Session 4, more in Sessions 10, 12–14 compared with Session 5 or 7 and in Sessions 9–14 compared with Session 6. Increases in drug intake were also confirmed within the Methylone-upper group with more drug infusions being obtained in the 14^{th} session relative to Sessions 1–7, 9, 12, more in Session 13 compared with Sessions 1–5, more in Session 8 or 10 compared with Sessions 1,3–4 and there were also more infusions obtained in Sessions 6, 11–12 compared with the first session. The Mephedrone-lower group only obtained significantly more infusions in the 14^{th} session compared with Sessions 1–3 and in the 13^{th} session compared with Session 1. The post hoc test did not confirm any significant differences across the 14 sessions for either MDMA-upper, MDMA-lower or Methylone-lower groups.

3.2.2 Drug-Lever Discrimination—Drug-associated lever discrimination (Figure 3, **middle and lower panels**) for the median-split groups (defined by the infusions, above) was also significantly affected by training Session [$F(13, 533) = 9.58$; $P < 0.0001$] and by Group [$F(5, 41) = 3.73$; $P < 0.01$] but not by the interaction [$F(65, 533) = 0.80$; $P = 0.86$]. The post-hoc test confirmed significant differences in discrimination ratio between the mephedrone-upper group and the MDMA-lower (Session 7) and Methylone-lower (Sessions 8, 11) groups. Within groups, the post hoc test only confirmed that the Mephedrone-upper group achieved significantly higher lever discrimination in Sessions 8–14 compared with their discrimination on Session 3. Follow up ANOVA conducted on the upper and lower halves of the distributions for the three drugs confirmed a main effect of session but not drug identity within both of the the upper [$F(13, 273) = 7.37$; $P < 0.0001$] and lower [$F(13, 260) = 3.91$; $P < 0.0001$] preference halves. Posthoc analysis of the marginal means confirmed

that lever discrimination was significantly higher in the 12th and 13th sessions relative to Sessions 1–3 for the lower-preference groups and significantly increased over Sessions 1 and 3 (Sessions 10–14) and Session 2 (Session 10–11, 13–14) compared with in the upper-preference groups.

3.3 FIXED RATIO DOSE SUBSTITUTION

3.3.1 Mephedrone-trained and MDMA-trained rats—Approximately equivalent numbers of individuals from the upper and lower halves of the acquisition distribution completed the FR dose-response series with patent catheters. For the mephedrone-trained rats completion was as follows: Mephedrone N=15 (8 from upper acquisition half), Methylone N=10 (5 upper half), MDMA N= 9 (5 upper half) and for the final mephedrone N=9 (5 upper). MDMA-trained rats that remained patent through the FR series were as follows: MDMA N=15, (8 upper half), Methylone N= 14 (7 upper half), 4-MMC N=13 (6 upper half) and for the final MDMA N=11 (5 upper half).

In the first FR dose substitution with their respective training drugs (Figure 4A), the ANOVA confirmed significant effects of dose [$F(4, 112) = 29.93$; $P < 0.0001$], of Group/Drug [$F(1, 28) = 14.77$; $P < 0.001$] and the interaction [$F(4, 112) = 9.717$; $P < 0.0001$] on mean infusions obtained. Significant differences between groups at the 0.0 and 0.25 mg/kg/inf conditions. Infusions obtained by the MDMA-trained animals only differed between 0.0 and 2.5 mg/kg/inf doses. For the Mephedrone-trained group, significant differences in infusions were confirmed between each of the vehicle and 0.25 mg/kg/inf as compared with the 0.5–2.5 mg/kg/inf conditions, as well as between the 0.5 and 2.5 mg/kg/inf conditions.

Analysis of the drug-lever discrimination confirmed a significant effect of dose [$F(4, 112) = 5.64$; $P < 0.0005$] and of group [$F(1, 28) = 7.08$; $P < 0.05$]. The posthoc test confirmed that across the groups, discrimination was lower in the saline (68%) versus active dosing conditions (81–85%). The posthoc test also confirmed that when saline was available the MDMA-trained group's drug-lever discrimination (59%) was significantly lower than the mephedrone-trained group's discrimination (78%) but no differences in the active dose conditions between MDMA-trained (74–81%) and mephedrone-trained (81–92%) rats were confirmed.

In the second FR dose substitution in which both groups received doses of Methylone (Figure 4B), the analysis confirmed a significant effect of dose [$F(4, 88) = 12.74$; $P < 0.0001$] but not original training Group [$F(1, 22) = 3.954$; $P = 0.06$] or of the interaction [$F(4, 88) = 0.7995$; $P = 0.53$] on mean infusions obtained. Posthoc test confirmed that for the MDMA-trained group, infusions of methylone differed between the 0.25 mg/kg/inf condition and the 1.0 and 2.5 mg/kg/inf conditions. Within the Mephedrone-trained group, infusions significantly differed between the 2.5 mg/kg/inf condition and each of the 0.0, 0.25 and 0.5 mg/kg/inf conditions, as well as between the 0.25 and 1.0 mg/kg/inf conditions.

Analysis of the drug-lever discrimination confirmed a significant effect only of dose [$F(4, 88) = 4.35$; $P < 0.005$] and the posthoc confirmed that discrimination across both groups was lower in the saline condition (71%) compared with the active dose conditions (82–85%).

Comparison of the MDMA-trained group's curves during the first two dose-substitutions suggested the possibility that the dose range had not been extended sufficiently on the lower end to determine differential adjustment to MDMA dose. Thus a dose of 0.125 mg/kg/inf was incorporated for the final two studies and the 1.00 mg/kg/inf was omitted. In the drug-swap condition, MDMA-trained animals received doses of mephedrone and the mephedrone-trained animals received doses of MDMA (Figure 3C). The ANOVA confirmed a significant effect of dose [$F(4, 80) = 17.59$; $P < 0.0001$] but not training Group/Drug [$F(1, 20) = 0.08$; $P = 0.78$] or of the interaction [$F(4, 80) = 1.371$; $P = 0.25$] on mean infusions obtained. Posthoc test confirmed that for the MDMA-trained group, infusions of mephedrone differed between the 2.5 mg/kg/inf condition and the 0.125, 0.25 and 0.5 mg/kg/inf conditions. Within the mephedrone-trained group, infusions significantly differed between the 0.125 mg/kg/inf condition and each of the 0.0, 0.5 and 0.25 mg/kg/inf conditions. Fewer infusions of MDMA were obtained in the 2.5 mg/kg/inf condition compared with the 0.25 mg/kg/inf condition as well.

Analysis of the drug-lever discrimination ratios confirmed a significant effect of dose [$F(4, 80) = 5.62$; $P = 0.0005$] and the interaction of dose with group [$F(4, 80) = 4.12$; $P < 0.005$]. The posthoc test confirmed that discrimination in the mephedrone-trained group (testing on MDMA) was lower in the saline condition (64%) compared with the active dose conditions (72–88%) and lower in the 2.5 mg/kg/inf condition (72%) compared with the 0.125 mg/kg/inf condition (88%). No differences in drug lever discrimination were confirmed of the MDMA-trained goup (tested on mephedrone).

In the final FR dose response series the animals were returned to their original training drug (Figure 4D). The analysis confirmed a significant effect of dose [$F(4, 72) = 22.27$; $P < 0.0001$] but not Group/Drug [$F(1, 18) = 1.72$; $P = 0.21$] or of the interaction [$F(4, 72) = 1.085$; $P = 0.37$] on mean infusions obtained. Posthoc test confirmed that for the MDMA-trained group, infusions of MDMA significantly differed between each of the 0.125 and 0.25 mg/kg/inf conditions as compared with each of the 0.0, 0.5 and 2.5 mg/kg/inf conditions. Significantly more infusions were obtained in the mephedrone-trained group in the 0.125 mg/kg/inf condition compared with every other dose condition and in the 0.25 mg/kg/inf condition compared with the 2.5 mg/kg/inf condition.

No significant effects drug or group were confirmed for the drug-lever discrimination ratios in this series.

3.3.2 Methylone trained rats—In this group (N=11; 6 upper half of acquisition distribution) the dose ranges were identical for four sequential dose- substitution studies (Figure 5). The repeated measures ANOVA confirmed a significant effect of dose [$F(4, 40) = 12.05$; $P < 0.0001$] but not of drug identity [$F(3, 30) = 1.14$; $P = 0.35$] nor any interaction of factors [$F(12, 120) = 1.02$; $P = 0.44$]. Post hoc analysis (Tukey) confirmed significant differences between the 0.125 and 2.5 mg/kg/inf doses in all four dose-substitution series and between the 0.25 and 2.5 doses in the mephedrone, MDMA and repeated methylone series. There was also a significant difference in infusions obtained between the 0.5 mg/kg/inf and 2.5 mg/kg/inf mephedrone doses and between vehicle and the 0.125 and 0.25 mg/kg/inf doses of the final methylone series.

No significant effects drug or group were confirmed for the drug-lever discrimination ratios in this series.

3.4 PROGRESSIVE RATIO DOSE SUBSTITUTION

As with the FR series, approximately equivalent numbers of individuals from the upper and lower halves of the original acquisition distributions for all three drugs completed the PR dose-response series with patent catheters. This included the mephedrone-trained (N=9 ; 5 upper half of acquisition distribution), MDMA-trained (N=10; 6 upper half) and methylone-trained (N=8, 4 upper half) groups.

The one-way ANOVA analysis of breakpoint in the Mephedrone-trained group (Figure 6) confirmed a significant effect of dose [$F(1, 8) = 8.29$; $P < 0.05$] but not of drug identity [$F(2, 16) = 0.19$; $P = 0.83$] or the interaction [$F(2, 16) = 0.18$; $P = 0.84$]. The posthoc test confirmed that significantly higher breakpoints were reached with the 1.0 mg/kg/inf dose relative to the 0.125 mg/kg/inf dose condition for each drug. The one-way ANOVA analysis of breakpoint in the MDMA-trained group did not confirm any significant effect of dose [$F(1, 10) = 3.85$; $P = 0.08$], of drug identity [$F(2, 20) = 0.72$; $P = 0.50$] or the interaction [$F(2, 20) = 0.51$; $P = 0.61$]. Similarly, the one-way ANOVA analysis of breakpoint in the methylone-trained group did not confirm any significant effects of dose [$F(1, 7) = 1.683$; $P = 0.24$] of drug identity [$F(2, 14) = 0.90$; $P = 0.4287$] or the interaction [$F(2, 14) = 0.2722$; $P = 0.77$].

Analysis of the drug-lever discrimination ratios confirmed a significant effect of drug identity [$F(2, 20) = 3.62$; $P < 0.05$] for MDMA-trained animals in the PR test, but posthoc testing failed to confirm individual differences in mean discrimination ratio between the MDMA (78%), mephedrone (85%) and methylone (86%) drug challenges. No significant effects were confirmed for the drug-lever discrimination ratios for mephedrone-trained or methylone-trained rats.

4. DISCUSSION

Prior to this investigation there were only limited data available on the IVSA of novel cathinone stimulant drugs which confer the atypical neuropharmacological profile associated with MDMA such as mephedrone (Aarde et al., 2013a; Hadlock et al., 2011; Motbey et al., 2013) and methylone (Watterson et al., 2012) and the comparison was necessarily indirect. In the present study, the first *direct* comparison of the abuse liability of atypical, empathogenic stimulant drugs (i.e., those that preferentially enhance serotonin versus dopamine accumulation in the nucleus accumbens), it was found that mephedrone (4-methylmethcathinone) is more effective as a reinforcer than either MDMA or methylone. This was observed first in the initial *acquisition* of intravenous self-administration (IVSA) since more infusions were obtained by rats trained on mephedrone than on either other drug and mephedrone-trained animals directed a higher proportion of presses on the drug-associated lever. The data are also consistent with the conclusion that a history of mephedrone IVSA established a lasting group difference in propensity to self-administer (stimulant) drugs, independent of specific identity. For example, the mephedrone-trained group acquired as many infusions of the lowest per-infusion dose of MDMA in the FR

procedure as they did of the lowest dose of mephedrone and more than either other group earned. Even more tellingly, the Progressive Ratio test found that the mephedrone-trained group was differentially sensitive to drug dose across all three compounds, generating significantly higher breakpoints at the higher dose, whereas the other groups had only moderately higher breakpoints at the higher doses. The mephedrone-trained group also exhibited no differentiation between the three compounds on the PR. This direct comparison of drugs was similar in outcome to the *indirect* comparison across the FR dose-substitutions in that group.

The median split analysis further demonstrated that the enhanced reinforcing value of mephedrone extended across the individual preference distribution. Indeed, the lowest-preferring mephedrone-trained animals matched the intake of the highest preferring animals trained on MDMA across the acquisition interval. There was also an indication in this analysis that significantly more methylone was self-administered than was MDMA for the higher-preferring animals and that furthermore, there was a significant increase in drug intake across training sessions for the upper half of the methylone group not observed in the upper half of the MDMA group. This was the only clear indication in the study of differential abuse liability between methylone and MDMA, however the size of the effect was modest. Although loss of catheter patency across the dose-substitution studies reduced sample sizes below that necessary for comparison between the halves of each acquisition distribution, approximately equal numbers of higher- and lower-preference animals were maintained. Thus it is unlikely that differential individual preferences within the training groups contributed to the observed results in the FR and PR dose-substitution testing.

Interpretation from the acquisition data alone is somewhat limited by the selection of a single training dose of each compound. It is not impossible that a lower potency drug would lead to increased intake relative to a higher potency drug such as the initial comparison of mephedrone self-administration with methamphetamine (Hadlock et al., 2011). The results of the dose-substitution studies are therefore critical to the overall conclusion. The FR test within the mephedrone-trained rats reflected a tremendous similarity of potency and efficacy across the three drugs. The MDMA-trained and methylone-trained groups, conversely, showed some evidence of learning across the four FR dose-substitution series which complicates interpretation of efficacy, particularly of their first FR series with their respective training drugs. It is unknown if four sequential dose substitutions produced greater sensitivity to doses in the final assessment or if the mephedrone FR test may have had a specific effect, perhaps on subsets of individuals. Nevertheless, if this phenomenon reflects some ongoing behavioral acquisition in these groups across the FR series, then this supports the overall conclusion that mephedrone was more effective in the acquisition phase. The similarity of the behavior reinforced by all three drugs *within* training groups in both the FR and PR procedures suggests that the difference in the original acquisition behavior was probably not due to selection of training doses with substantially different potencies. This further enhances confidence that mephedrone was a superior reinforcer for the original acquisition of IVSA.

The scientific literature has provided some initial clues as to distinctions between individual entities in a handful of initial reports on the pharmacological (Baumann et al., 2012;

Baumann et al., 2013; Simmler et al., 2013; Simmler et al., 2014) and even locomotor / subjective properties of the substituted cathinone stimulants, including mephedrone and methylone. For example, Simmler and colleagues report a DAT/SERT ratio on transporter inhibition of 0.08 for MDMA, 1.4 for mephedrone and 3.3 for methylone. DAT inhibition potency (IC_{50}) was 17 for MDMA, 4.82 for methylone and 3.31 for mephedrone which does not support the IVSA grouping of MDMA and methylone. A better prediction might be found in monoamine release mediated by the DAT where the EC_{50} for mephedrone 3.75 uM, for MDMA was 22 uM but it was reported as >100 uM for methylone. MDMA and mephedrone were actually about equipotent at inhibiting serotonin release mediated by the SERT (5.63 uM, 5.98 uM, respectively) however the EC_{50} for Methylone was >10 uM. These parameters likewise fail to explain the outcome of this IVSA assessment. An analysis of transporter mediated monoamine release in rat synaptosomes (Baumann et al., 2012) reported DAT/SERT ratios (mephedrone 2.41; methylone 1.82; MDMA 0.97) that are the best predictors of the present IVSA data. Nevertheless, since methylone and MDMA grouped in the present study IVSA potency is not a quantitative reflection of even the DAT/SERT ratios derived even from rat synaptosomes and a threshold model might be a better predictor. A final consideration may lie in the estimate of lipophilicity provided by Simmler et al 2013. In an epithelial model of blood-brain barrier permeability it was found that MDMA and methylone were about equivalent whereas the permeability estimate for mephedrone was ~70–150% greater; this may possibly explain the greater effectiveness of mephedrone in reinforcing IVSA behavior.

The mephedrone data are consistent with prior observations that IVSA is readily established and that this substituted cathinone is no less effective than methamphetamine in male rats (Aarde et al., 2013a; Hadlock et al., 2011; Motbey et al., 2013). The findings do not align with the report of Watterson (Watterson et al., 2012) with respect to methylone, however those prior results were in male rats so it is possible that a sex difference explains the disconnect with the present methylone results. Additional methodological differences of note include the strain (Sprague Dawley vs Wistar) and the precise training schedule (7 versus 5 days per week). Given the broader literature on stimulant drug self-administration in rats, it is unclear that these factors would result in a difference as striking as the contrast of the present methylone data with the finding of Watterson and colleagues. Since we used the most behaviorally effective dose used in that prior paper, it is unlikely that training dose explains the difference. The MDMA IVSA data are the first available for female rats, but the diverse outcome in prior studies using male rats (Ball et al., 2007; Dalley et al., 2007; De La Garza et al., 2007; Feduccia et al., 2010; Schenk et al., 2012; Schenk et al., 2007) makes it impossible to determine whether sex differences are apparent.

The present findings also contrast with some, but not all, other behavioral findings. Mephedrone and methylone produce similar dose-response functions for locomotor stimulation in mice and identical potencies on a drug-discrimination assay when rats are trained on cocaine; however methylone was less potent in methamphetamine-trained rats (Gatch et al., 2013). Bonano and colleagues concluded on the basis of relatively lower potency to decrease intracranial self-stimulation reward (ICSS) thresholds (Bonano et al., 2013) that mephedrone would have reduced abuse liability compared with methylone; the present results in a more specific behavioral assay suggest the opposite. Interestingly,

however, Watterson and colleagues found that methylone produced small, statistically unreliable, decreases in rat ICSS thresholds across a wide dose range (0.1–10 mg/kg, i.p.) but Robinson and colleagues (Robinson et al., 2012) found mephedrone to be as potent as cocaine in reducing mouse ICSS thresholds. The frequency-rate curves for R- or racemic mephedrone exhibit biphasic effects at higher doses that are not observed to the same extent with methylone, MDMA or at all with several other monoamine releasers (Bauer et al., 2013; Bonano et al., 2013; Gregg et al., 2014). Given differences in the ICSS findings to date, the relative paucity of direct comparisons between drugs in an identical model and large qualitative differences in the frequency-rate-dose curve relationships it is premature to propose reasons for why the present IVSA data might differ from some, but not all, prior ICSS findings.

In summary the IVSA data resulting from this study are consistent with a conclusion that mephedrone has relatively high abuse liability whereas methylone has a comparatively lower liability for repetitive use that is commensurate with that of MDMA. Importantly, these data show that inferences about reinforcer efficacy and abuse liability based on structure alone are not sufficient. Furthermore, inferences based on several *in vitro* pharmacological properties, and even *in vivo* neurochemistry (Baumann et al., 2012), may also be insufficient to predict abuse liability. Thus, it is imperative to assess novel stimulant drugs of abuse in well established *in vivo* model systems to determine likely implications for human health.

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Highlights

- Methylone and mephedrone exhibit MDMA-like neurochemical properties
- Intravenous self-administration of MDMA is inconsistent
- Mephedrone was a more effective reinforcer than MDMA or methylone
- Within training groups, all three drugs were of identical efficacy and potency
- Mephedrone-trained rats self-administered more than the other groups

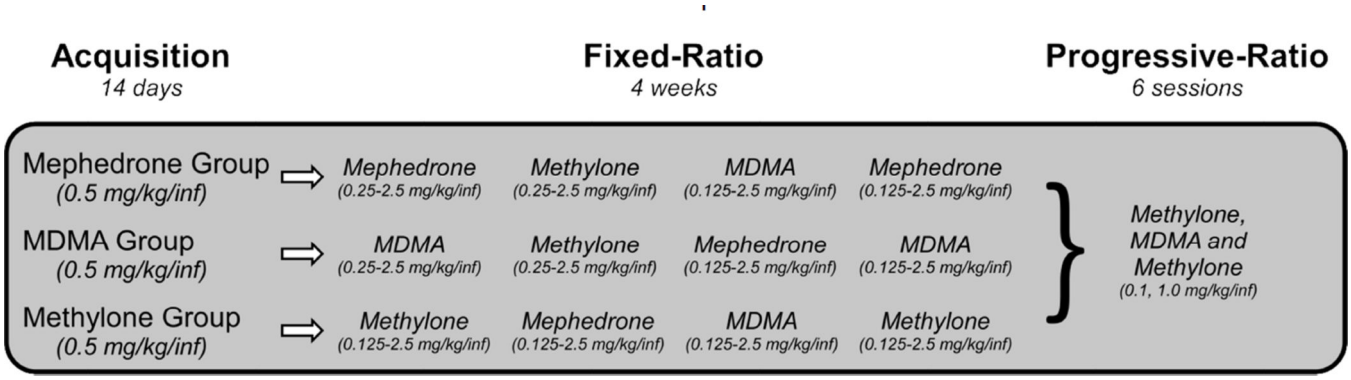

Figure 1.

A schematic depicting the sequential treatment phases for the experimental groups originally trained to self-administer Mephedrone, MDMA and Methylone.

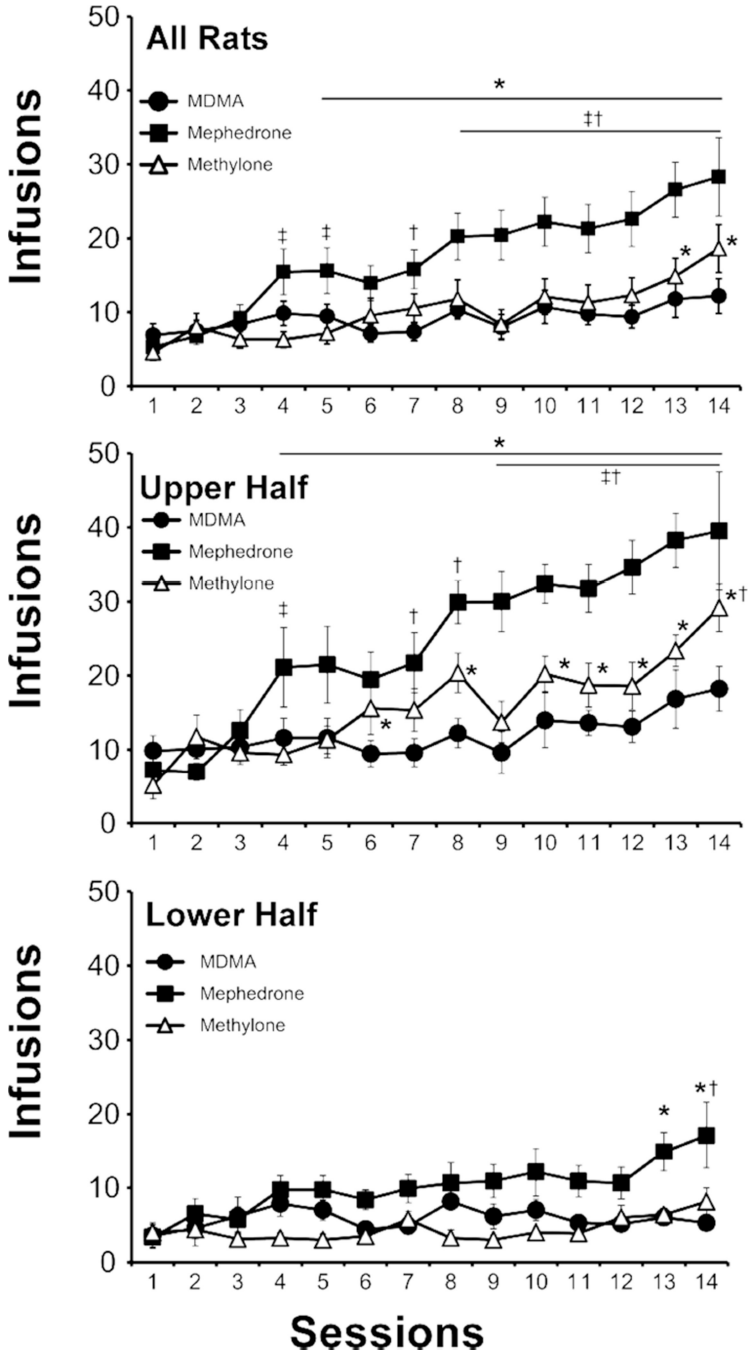

Figure 2.

Mean (±SEM) infusions obtained during acquisition (upper panel) are presented for all animals in the three groups (upper panel) as well as for the upper (middle panel) and lower (lower panel) median halves. Significant difference from the first session within group by *, Between mephedrone and both other groups by #, versus methylone by ‡ and versus MDMA by †.

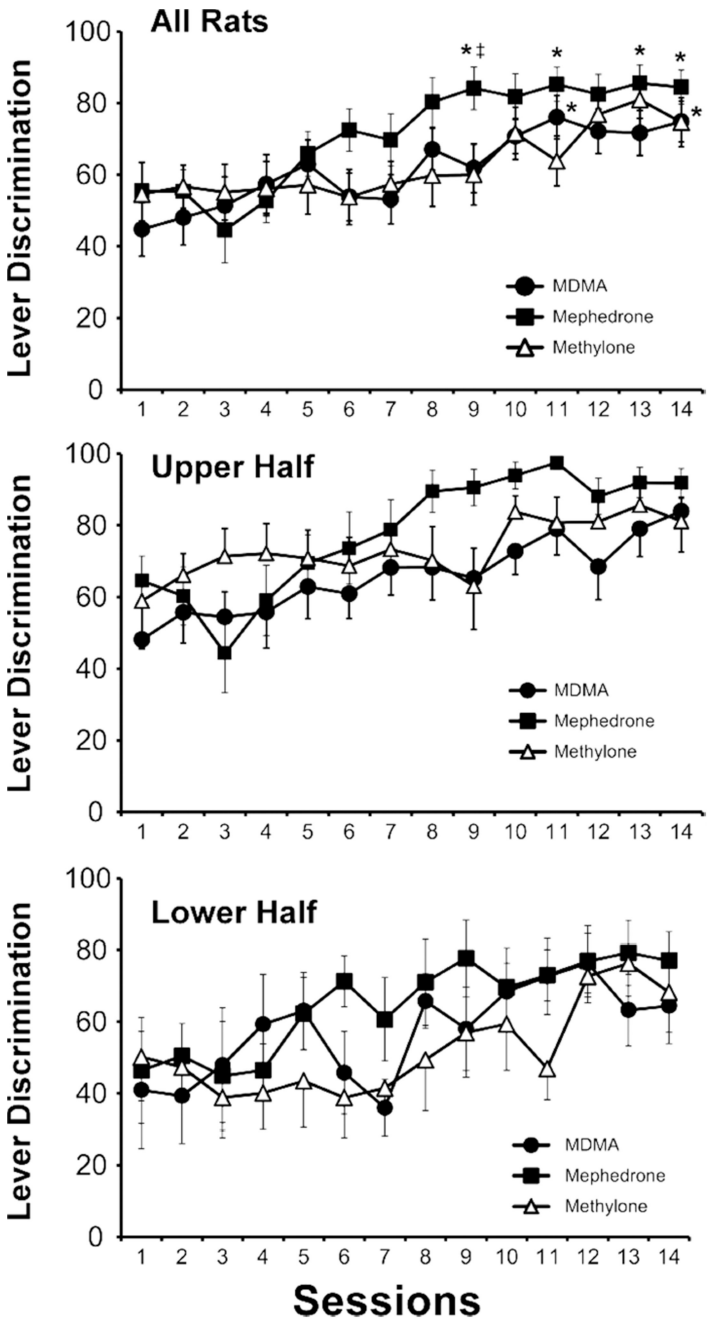

Figure 3.

Mean drug-associated lever discrimination ratios during acquisition are presented for all animals in the three groups (upper panel) as well as for the upper (middle panel) and lower (lower panel) median halves. Significant difference from the first session within group by *, Between mephedrone and both other groups by #, versus methylone by ‡ and versus MDMA by †

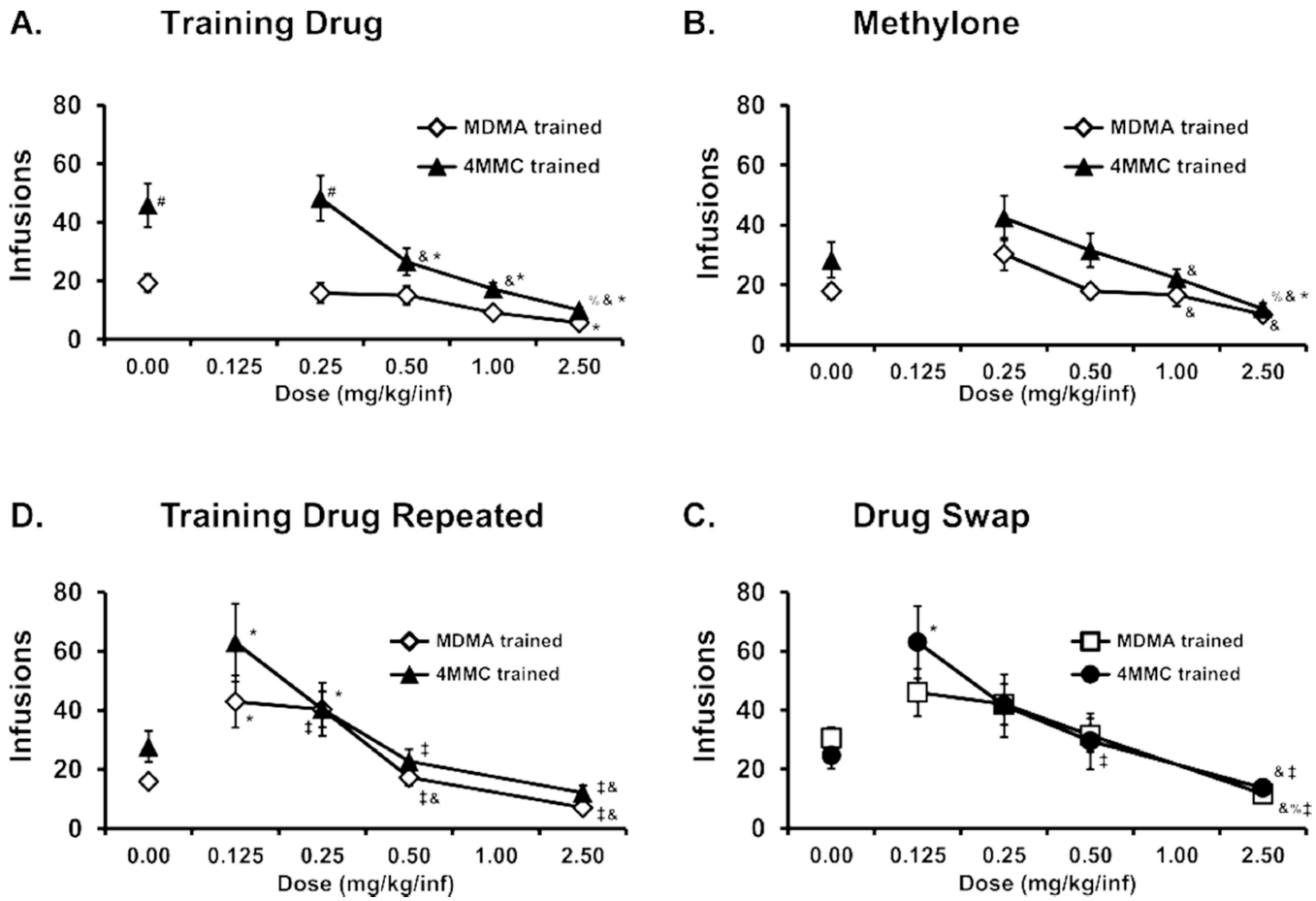

Figure 4.

Mean (±SEM) infusions obtained during dose-substitution in the MDMA-trained and Mephedrone-trained rats (N=9–15, see text) under a fixed-ratio 1 response requirement. The groups completed a dose series in the order of A) Their training drug; B) Methylone; C) MDMA-trained on mephedrone, mephedrone-trained on MDMA and D) Their original training drug. Significant differences between the training groups are indicated by #. Significant differences from the vehicle condition are indicated by *, from the 0.125 mg/kg/inf condition by ‡, from the 0.25 mg/kg/inf condition by & and from the 0.5 mg/kg/inf is indicated by %.

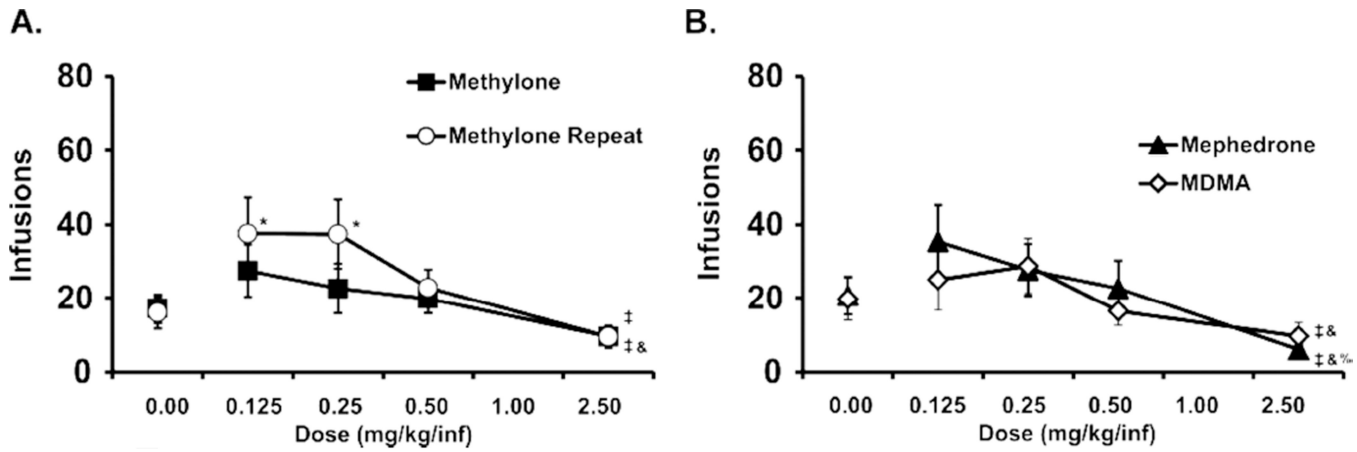

Figure 5.

Mean (±SEM) infusions obtained during dose-substitution in the Methylone-trained rats (N=11) under a fixed-ratio 1 response requirement. The group completed dose series in the order of Methylone (A), Mephedrone (B), MDMA (B) and Methylone repeated (A). Significant differences from the vehicle condition are indicated by *, from the 0.125 mg/kg/inf condition by ‡, from the 0.25 mg/kg/inf condition by & and from the 0.5 mg/kg/inf is indicated by %.

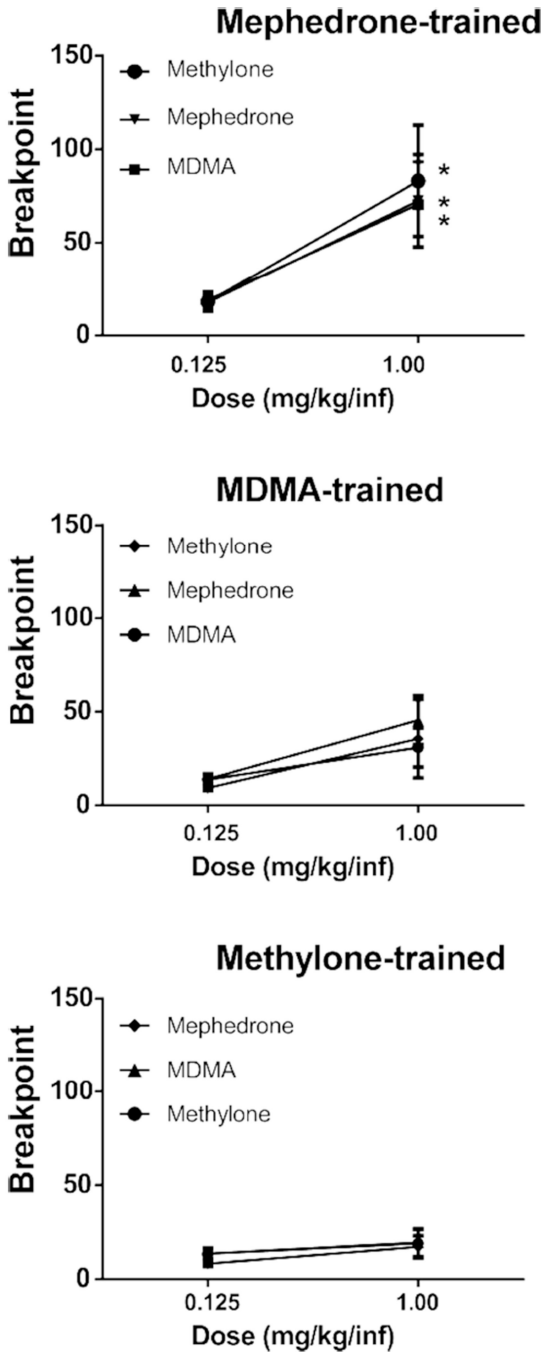

Figure 6.

Mean (±SEM) infusions obtained during dose-substitution in the MDMA-trained (N=10) Mephedrone-trained (N=9) and Methylone-trained (N=8) rats under a Progressive-Ratio schedule of reinforcement. Significant differences between doses, within drug, are indicated by *.