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Discriminative Stimulus Properties of 3.0 mg/kg Mephedrone in Rats

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Abstract

Consumption of a prominent synthetic cathinone known as mephedrone (4-methylmethcathinone) has become a popular alternative to club drugs such as ecstasy, cocaine, and methamphetamine within the past decade. The pharmacological mechanisms that contribute to its subjective effects have yet to be fully characterized and are thus warranted for investigation. The present study employed drug discrimination methods to train eight male Sprague-Dawley rats to discriminate injections of 3.0 mg/kg mephedrone from saline. Various doses of mephedrone, 3,4-methylenedioxypyrovalerone (MDPV), *d*-amphetamine (AMPH), cocaine, (+)-methamphetamine (METH), and 3,4-methylenedioxy-methamphetamine (MDMA) were investigated for their ability to substitute for the stimulus cues of the training drug. Full substitution was attained from 1.0 and 3.0 mg/kg mephedrone as well as 3.0 mg/kg MDMA. MDPV, AMPH, cocaine, and METH all produced partial substitution for the training stimulus. These data suggest that the interoceptive stimulus cues produced by mephedrone at 3.0 mg/kg may be predominantly mediated by serotonergic mechanisms. Future investigations should employ similar methods to perform substitution tests with monoaminergic receptor agonists and antagonists. This study contributes a unique investigation into the pharmacological basis of mephedrone's *in vivo* actions and provides data for interpretation.

Keywords: behavioral pharmacology, drug discrimination, synthetic cathinone, mephedrone

Discriminative Stimulus Properties of 3.0 mg/kg Mephedrone in Rats

Mephedrone (4-methylmethcathinone, 4-MMC) is a psychoactive drug reported to produce stimulant-like effects on the central nervous system (CNS) that most closely resemble the subjective effects of MDMA or ‘Ecstasy’ (Carhart-Harris, King, & Nutt, 2011). It belongs to a group of drugs known collectively as synthetic cathinones. These substances are structurally analogous to cathinone, a monoamine alkaloid found in the leaves of Khat (*C. edulis*), a native plant in the Horn of Africa and the Arabian Peninsula. Khat leaves are frequently chewed by populations in the Middle East for their CNS stimulant effects. Cathinone is believed to be the active compound responsible for these effects, and to a lesser extent, cathine which is also found in the leaves of Khat (European Monitoring Centre for Drugs and Drug Addiction, 2015).

Synthetic cathinones appeared on the recreational drug market during the mid-2000’s. They were sold under false monikers such as “Bath Salts”, “Plant Food”, and “Research Chemicals” and labeled as “not for human consumption” in an attempt to circumvent legislative regulations in Europe and the United States. Mephedrone, MDPV (3,4-methylenedioxypyrovalerone), and methylone (3,4-methylenedioxy-N-methylcathinone) have been the most common constituents of many synthetic cathinones sold within the United States and Europe (German, Fleckenstein, & Hanson, 2013). Appeal for these substances had initially skyrocketed largely because there were no regulations banning their manufacture and distribution. Thus, the uncontrolled status provided drug users with a cheap and quasi-legal alternative to popular ‘club drugs’ like ecstasy, cocaine, and amphetamines. A drug use survey conducted in 2010 found that 54.7% of 2,200 respondents had consumed at least one of the aforementioned synthetic cathinones (Dick & Torrance, 2010; Capriola, 2013). In 2011, poison control centers in the US had recorded 6,138 calls regarding ‘bath salt’ exposure (American

Association of Poison Control Centers, 2016). These substances eventually gained control status throughout a large portion of Europe in 2010 and were placed under Schedule I of the Controlled Substances Act in 2012 by the United States. Most synthetic cathinones are believed to be manufactured primarily in China and in neighboring locations throughout Southeast Asia (US Department of Justice, 2011).

Some of the desired effects of these drugs are increased energy, heightened sex drive, euphoria, and openness which are outlined in a review by Prosser and Nelson (2011). However, adverse side effects resulting from the consumption of drugs like mephedrone and MDPV are certainly present. Regarding mephedrone specifically, some of its reported adverse side effects include paranoia, bruxism, hyperthermia, tachycardia, headaches, and agitation (Prosser & Nelson 2011; World Health Organization, 2014). In a survey of 205 mephedrone users (Dargan, Albert, & Wood, 2010), 17.6% of respondents indicated symptoms of addiction, although the World Health Organizations Expert Committee on Drug Dependence (2014) reported the addictive properties to be primarily of psychological origin (Dargan, Albert, & Wood, 2010). The widespread use of mephedrone, its association with adverse side effects, and the potential compulsive use leading to addiction warrant investigations into its pharmacology.

Currently, mephedrone is considered to predominantly affect monoaminergic neurotransmitter systems. Microdialysis experiments in rats have demonstrated that mephedrone administered at doses of 0.3 and 1.0 mg/kg produce acute increases in extracellular concentrations of serotonin (5-HT), dopamine (DA), and norepinephrine (NE) with a slight preference towards 5-HT release (Baumann et al., 2012). Furthermore, mephedrone administered at a dose of 3.0 mg/kg was shown to increase extracellular concentrations of DA and 5-HT in the rat nucleus accumbens to approximately 496% and 941% above baseline levels, respectively

(Kehr et al., 2011). These findings along with separate observations that mephedrone induces conditioned place preference (CPP) in both rats and mice (Karlsson, Andersson, Kronstrand, & Kugelberg, 2014; Lisek et al., 2012) indicates a potential risk for addiction. Baumann et al. (2012) confirmed that mephedrone acts as a nonselective substrate for serotonin transporters (SERT), dopamine transporters (DAT), and norepinephrine transporters (NET). It is believed that mephedrone induces the release of such monoamines by entering presynaptic buttons through membrane transporters, thereby causing an efflux of neurotransmitters due to an increase in cytosolic transporter substrate. Further experiments have observed mephedrone to produce a depolarizing current at human DAT expressed in *Xenopus* oocytes, similar to other dopamine releasing agents such as amphetamine (Cameron, Kolanos, Verkariya, Felice, & Glennon, 2013). Mephedrone also acts as a 5-HT and DA reuptake inhibitor with a greater affinity towards DA transporters (Martinez-Clemente, Escubado, Pubill, & Camarasa, 2012; Hadlock et al., 2011). It is noteworthy that enantiomeric differences exist in the actions of mephedrone. The S-isomer of mephedrone produces a substantially greater effect on the reuptake inhibition and release of 5-HT when compared to R-mephedrone and racemic mixtures, while all three forms do produce increases in DA and 5-HT (Gregg, Baumann, Vouga, Tallarida, & Rawls, 2015).

Of particular interest to the current study are the interoceptive stimulus cues produced by mephedrone, and the extent to which these cues are similar to those produced by substances like amphetamine or MDMA. Behavioral pharmacology research commonly employs drug discrimination procedures to compare the interoceptive stimulus effects of novel drugs to known drugs of abuse. A detailed description of drug discrimination methods is provided in a text devoted to this approach by Glennon and Young (2011). Briefly, subjects are trained to discriminate between the stimulus cues of a drug and the absence of those cues (e.g., saline

injections) by means of training a behavioral response (e.g., left lever press) following a drug injection and training a different behavioral response (e.g., right lever press) after a saline injection. Once animals have attained reliable responding under the foregoing training conditions, other drugs can be tested for stimulus generalization. It is generally assumed that if a test agent produces responding similar to the training drug ($\geq 80\%$ drug-lever selection), then the stimulus effects of the two drugs may produce similar interoceptive effects. It is important to emphasize that the stimulus effects produced by a particular training drug will vary with its dose (Stolerman, Childs, Ford, & Grant, 2011). Nevertheless, drug discrimination methods provide a means for quantitatively assessing the interoceptive stimulus effects of a pharmacologically-active agent in comparison to other compounds. Such methods are often used to provide insight as to how an agent functions *in vivo*, as stimulus cues are often reflective of events occurring at the neuronal level. These methods can also be used to test the substitution of specific neuronal receptor antagonists and agonists with certain drugs.

To date, only four published studies have examined synthetic cathinones using drug discrimination methods (Varner et al., 2013; Fantegrossi, Gannon, Zimmerman, & Rice, 2013; Gatch, Taylor, & Forster, 2013; Harvey and Baker, 2016). Varner et al. (2013) trained rats to discriminate 3.2 mg/kg mephedrone from saline and observed a dose-dependent increase in mephedrone-like responding with MDMA and two prototypical stimulants (cocaine, methamphetamine), and little to no substitution with non-stimulants (fenfluramine, morphine, and phencyclidine). However, only MDMA was reported to fully substitute for mephedrone in this study. In contrast, Gatch et al. (2013) observed full substitution with mephedrone as well as MDPV in rats trained to discriminate 10 mg/kg cocaine or 1 mg/kg methamphetamine. In another study, mice were trained to discriminate MDPV from saline at a dose of 0.3 mg/kg and

showed complete generalization with MDPV, MDMA, and methamphetamine (Fantegrossi et al., 2013). More recently, both mephedrone and MDPV were shown to fully substitute in rats trained to discriminate a mixture of 1.5 mg/kg MDMA and 0.5 mg/kg amphetamine (Harvey and Baker, 2016). Mephedrone also produced full substitution in rats trained to discriminate 1.5 mg/kg MDMA, but MDPV produced only partial substitution in these animals. The collective results of the aforementioned studies indicate the interoceptive stimulus effects of mephedrone are most similar to those of MDMA, with some similarities between mephedrone and other psychostimulants. In an effort to further evaluate the pharmacological mechanisms contributing to mephedrone discrimination, the present study assessed psychoactive drugs with variable neurochemical actions in rats trained to discriminate 3.0 mg/kg mephedrone from saline. It was predicted that drugs that are predominantly 5-HT releasers (e.g., MDMA) would produce full substitution for mephedrone as previously observed by Varner et al. (2013). In contrast, it was expected that psychostimulants that are predominantly DA releasers (amphetamine, methamphetamine) or DAT inhibitors (cocaine, MDPV) would produce only partial substitution.

Methods

Subjects: Eight experimentally naïve adult male Sprague-Dawley rats (285-360g) were used in the present study. Rats were singly housed in a room maintained at 20°C (\pm 2°C) and 50% humidity (\pm 5%) and on a 12 h:12 h light/dark schedule with lights on between 0700 and 1900 hours. Rats had unlimited access to water in home cages and were fed a commercial rodent diet (Purina®, Richmond, Indiana, USA) in daily rations to maintain body weight at approximately 85% of free-feeding weights. Experimental procedures were performed between approximately 1400 and 1900 hours. All procedures were conducted in accordance with the *Guide for the Care*

and Use of Laboratory Animals: 8th ed. (National Research Council, 2011) and received approval from the Western Michigan University Institutional Animal Care and Use Committee.

Drugs: (±)-Mephedrone-hydrochloride, 3,4-methylenedioxypyrovalerone-hydrochloride (MDPV), 3,4-methylenedioxy-methamphetamine-hydrochloride (MDMA), cocaine-hydrochloride (COC), and (+)-methamphetamine-hydrochloride (METH) were provided by the National Institute on Drug Abuse Drug Control Supply Program (Bethesda, MD, USA). *d*-Amphetamine-hemisulfate was purchased from Sigma Chemical Company, Inc. (St. Louis, MO, USA). Each drug solution was prepared by dissolving the salt in 0.9% (wt/vol) bacteriostatic sodium chloride. Drug administration was performed via intraperitoneal (IP) injections using 1 mL syringes with a pre-session injection interval of 15 minutes.

Apparatus: Eight standard operant conditioning chambers (ENV-001; MED Associates Inc., St. Albans, VT, USA) were equipped with three retractable levers on the front panel, a food pellet dispenser, 28-V house lamp, and a fan for ventilation. Each chamber was housed within light and sound attenuating shells. Experimental events were programmed and controlled using Version IV Med-PC software (MED Associates Inc., St. Albans, VT, USA). Reinforcers used were 45 mg food pellets (Bioserv; Frenchtown, NJ, USA).

Preliminary Training Procedures: Subjects were acclimated to the operant conditioning chambers during a single 60 min period in which food pellets were delivered on a variable time 60 sec schedule. All levers were retracted during this session. Preliminary lever press training was then conducted during daily 20 min training sessions with the center lever extended. Lever presses were initially reinforced on a fixed ratio 1 (FR1) schedule that was gradually incremented to an FR 20 schedule over 5-7 sessions. Subsequently, errorless training sessions were conducted in which only the left lever or the right lever was extended and animals were

reinforced under an FR 20 schedule. Subjects were administered a 0.9% saline solution (vehicle, V) during the first two sessions of errorless discrimination training which lasted 20 minutes each. The next two sessions proceeded with the opposite lever extended in each chamber and after administering the training drug of 3.0 mg/kg mephedrone (D). This method proceeded with an additional V session, and one additional D session. Four rats were reinforced for responding on the left lever during drug training sessions and the right lever during saline training sessions. Conditions were reversed for the remaining four rats.

Discrimination Training Procedures: Following preliminary training, discrimination training sessions commenced with both left and right levers extended. Training sessions were conducted five to six days per week for 20 min per day. Rats were injected with 3.0 mg/kg mephedrone or saline in a pseudorandom order, with no more than two consecutive drug or saline sessions. Correct responses on the drug or vehicle lever were reinforced on an FR 20 resetting schedule. As such, subjects were required to emit 20 consecutive responses on the correct lever to receive a food pellet. Incorrect responses reset the counter for responses on the correct lever. Subjects were considered eligible to begin the testing phase if, for at least 8 out of 10 consecutive training sessions, the percentage of responses during the first FR and the percentage of responses for the total session were at least 80% on the injection-appropriate lever.

Stimulus Substitution Tests: During the first test session, subjects were administered a selected dose of a test drug and placed into operant chambers for up to 20 minutes. Test sessions were identical to discrimination training sessions with the exception that no food pellets were delivered and sessions ended upon completion of the first FR. Subjects were required to complete at least one D and at least one V session with 80% or higher injection appropriate responding before undergoing subsequent test sessions. Drugs tested included: mephedrone (0.1

- 3.0 mg/kg), MDPV (0.03 - 3.0 mg/kg), *d*-amphetamine (0.03 - 3.0 mg/kg), cocaine (1.0 - 10.0 mg/kg), (+)-methamphetamine (0.03 - 3.0 mg/kg), and MDMA (0.03 - 3.0 mg/kg).

Data Analysis: Results for the acquisition of stimulus discrimination were measured in the number of sessions it took for each subject to meet the criteria necessary to begin testing procedures. These data are expressed as a group mean ($\pm SEM$) with the range. Results for stimulus substitution tests are expressed as the mean ($\pm SEM$) percent mephedrone-lever (meph-lever) responding for each compound and dose tested. The rate of responding for each test session was plotted as the mean ($\pm SEM$) number of responses per second. Dose-response curves are included in Appendices A-C as a graphical representation of substitution and response rate data. Test response rates were statistically analyzed using a repeated measures analysis of variance (ANOVA) to determine whether doses had a significant effect on response rate. A Dunnett's multiple comparisons test identified which doses produced a significantly different rate of responding when compared to response rates during saline tests. For test agents that produced a mean of 80% or higher responding on the meph-lever, a non-linear regression analysis was performed to estimate effective dose (ED_{50}) values with a 95% confidence interval (CI). All statistical analyses were conducted using GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Discrimination Acquisition: Discrimination criteria were met within an average of 17.13 (± 2.69) sessions (range=12-32). Stimulus control remained stable throughout the experimental procedures.

Stimulus Substitution: Mephedrone, depicted in Figure A1, produced full substitution for the training stimulus at test doses of 1.0 mg/kg and 3.0 mg/kg and yielded an ED_{50} value of 0.43 mg/kg 95% CI [0.24, 0.75 mg/kg]. A repeated measures ANOVA indicated a significant effect of dose on response rate [$F(4,28) = 9.06, p < .0001$] with a multiple comparisons test identifying 3.0 mg/kg mephedrone to produce significantly different response rates than those observed during saline tests.

MDPV, depicted in Figure A2, produced partial substitution for mephedrone at 0.3 mg/kg and 1.0 mg/kg with a mean of 36% (± 16.4) and 46% (± 16.3) mephedrone-lever responding, respectively. A disruption of responding was observed at 3.0 mg/kg MDPV in six subjects. ANOVA results indicated a significant effect of dose on response rate [$F(5, 35) = 9.06, p < .0001$] for MDPV. A multiple comparisons test showed that response rates were significantly different from rates during saline tests at a dose of 3.0 mg/kg MDPV.

Amphetamine test data are depicted in Figure B1. Substitution tests revealed only partial substitution with a mean of 54% (± 14.5) at 1.0 mg/kg and 41% (± 11.7) at 3.0 mg/kg. Amphetamine test doses had a significant effect on response rate [$F(5, 35) = 14.02, p < .0001$] and completely suppressed responding in three subjects at 3.0 mg/kg. Both 1.0 and 3.0 mg/kg amphetamine produced response rates significantly different from rates during saline tests.

Cocaine, depicted in Figure B2, failed to substitute for mephedrone with only 37% (± 17.1) mephedrone-lever responses at 3.0 mg/kg and 50% (± 18.5) at 10.0 mg/kg. No significant effect of dose on response rate was observed for cocaine [$F(3,21) = 1.45, p = .256$].

Methamphetamine, depicted in Figure C1, also failed to engender stimulus generalization with 38% (± 15.9) mephedrone-lever responding at 1.0 mg/kg. Doses of 3.0 mg/kg

methamphetamine completely disrupted responding in all eight subjects. Significant effects of dose on response rate was observed for methamphetamine [$F(4,28) = 5.42, p = .0023$].

Methamphetamine at 1.0 mg/kg produced significantly different response rates than those observed during saline tests.

MDMA, depicted in Figure C2, produced full substitution for the training stimulus at 3.0 mg/kg with an ED_{50} value of 1.01 mg/kg 95% CI [very wide]. At 1.0 mg/kg, MDMA displayed partial substitution with a mean of 49% (± 18.7) mephedrone-lever responding. Dose had a significant impact on response rate [$F(5,35) = 13.69, p < .0001$]. MDMA at 1.0 and 3.0 mg/kg both produced rates of responding that were significantly different from those observed during saline tests.

Discussion

This study successfully established stimulus discrimination in rats using a training dose of 3.0 mg/kg mephedrone. Tests were performed using a range of doses of mephedrone, MDPV, amphetamine, cocaine, methamphetamine and MDMA to assess stimulus substitution to mephedrone. Results showed that 1.0 and 3.0 mg/kg mephedrone and 3.0 mg/kg MDMA were the only test doses to fully substitute for the stimulus effects of the training drug. These results are consistent with those of Varner et al. (2013), who reported MDMA produced full substitution in rats trained to discriminate 3.2 mg/kg mephedrone, whereas cocaine and methamphetamine only yielded partial substitution. Similarly, the current results demonstrated a dose-dependent increase in mephedrone-lever responding with cocaine, methamphetamine, and amphetamine, but did not approach full substitution. MDPV also displayed mephedrone-lever responding in a similar fashion showing partial substitution, except that the highest tested dose (3.0 mg/kg) approached an average of zero percent responding on the same lever. The highest tested doses of

MDPV, amphetamine, and methamphetamine yielded a suppression of responding in multiple subjects.

These data may suggest that the stimulus effects of mephedrone at the selected training dose are modulated principally by serotonergic mechanisms. Both amphetamine and methamphetamine display a preference for the reuptake inhibition and release of DA and NE over 5-HT (Fleckenstein, Gibb, & Hanson, 2000; Simmler et al., 2013). Similar to cocaine, MDPV has been observed as a potent DAT and NET blocker while producing little to no effect on the serotonin transporter (Simmler et al., 2013). Conversely, both mephedrone and MDMA at doses of 3.0 mg/kg cause an extracellular increase in 5-HT at roughly 900% above baseline levels with a much smaller effect on DA level increases (Kehr et al., 2011).

It is essential for the characterization of mephedrone's *in vivo* pharmacological properties that substitution tests involving receptor agonists and antagonists be conducted. Simmler et al. (2013) reported that mephedrone displays an affinity for 5-HT_{2A} and α_1 -adrenoreceptors among others. Additionally, the training dose of mephedrone used in this study should undoubtedly be altered in future investigations for potential comparisons. As previously mentioned, drug discrimination results are dependent on the dose of a training drug (Stolerman et al., 2011). A recreational dose of mephedrone typically ranges from 100-250 mg ("Mephedrone," n.d.). In a 170 lb. human, these quantities are equivalent to approximately 1 and 3 mg/kg. So the training dose employed in this study has social relevance yet lower training doses are certainly warranted for investigation.

Efforts to learn more about drug actions on the CNS are an invaluable part of addressing the global crisis of drug abuse and addiction. As the technological capability of synthesizing drugs becomes more easily accessible across the globe, new compounds will surely arise on the

recreational drug market. With this in mind, the need for pharmacological investigations of new and old substances cannot be overlooked. Synthetic cathinones are no longer novel substances, but their mechanistic actions are not yet fully understood. In order for health care professionals to adequately address cases involving substances like mephedrone, determined scientific inquisition of recreational drugs must not cease. This study offers a unique insight into a prominent synthetic cathinone and presents valuable data for interpretation.

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Appendix A

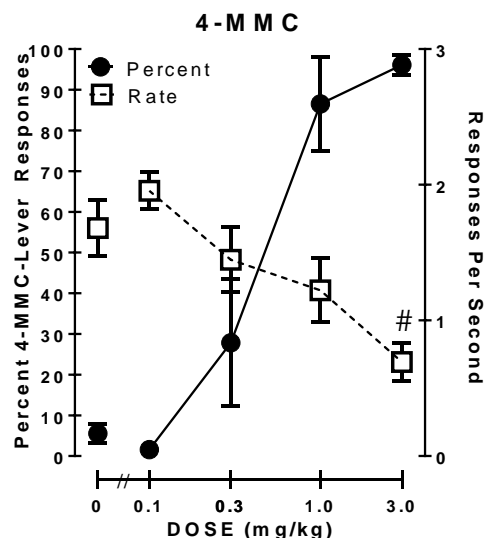


Figure A1. Mephedrone (4-MMC) dose-response curve in rats trained to discriminate 3.0 mg/kg mephedrone ($n=8$) from saline. Results are depicted as the mean ($\pm SEM$) percent 4-MMC-lever responses and the mean ($\pm SEM$) response rate in lever-presses per second. Symbols indicate response rates that significantly differed from rates observed during saline tests; $*p < .05$, $**p < .01$, $\#p < .001$, $##p < .0001$.

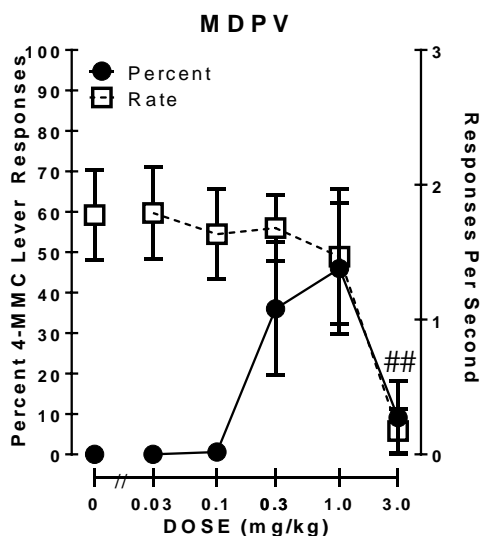


Figure A2. MDPV dose-response curve in rats trained to discriminate 3.0 mg/kg mephedrone ($n=8$, $n=2$ for % at 3.0 mg/kg) from saline. Results are depicted as the mean ($\pm SEM$) percent 4-MMC-lever responses and the mean ($\pm SEM$) response rate in lever-presses per second. Symbols indicate response rates that significantly differed from rates observed during saline tests; $*p < .05$, $**p < .01$, $\#p < .001$, $##p < .0001$.

Appendix B

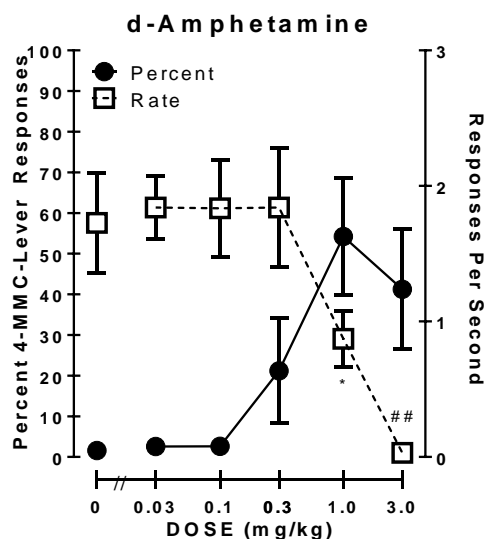


Figure B1. *d*-Amphetamine dose-response curve in rats trained to discriminate 3.0 mg/kg mephedrone ($n=8$, $n=5$ for % at 3.0 mg/kg) from saline. Results are depicted as the mean (\pm SEM) percent 4-MMC-lever responses and the mean (\pm SEM) response rate in lever-presses per second. Symbols indicate response rates that significantly differed from rates observed during saline tests; * $p < .05$, ** $p < .01$, # $p < .001$, ## $p < .0001$.

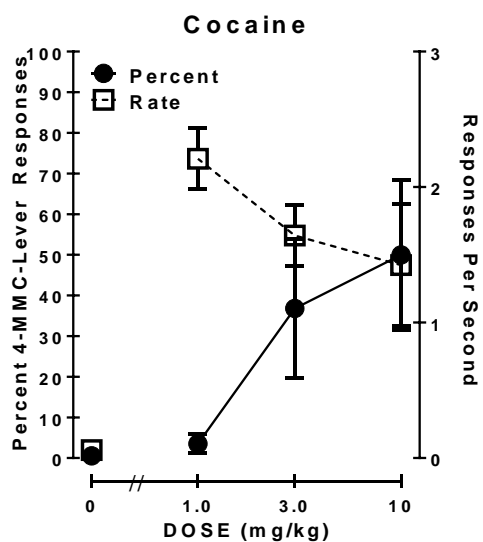


Figure B2. Cocaine dose-response curve in rats trained to discriminate 3.0 mg/kg mephedrone ($n=8$) from saline. Results are depicted as the mean (\pm SEM) percent 4-MMC-lever responses and the mean (\pm SEM) response rate in lever-presses per second.

Appendix C

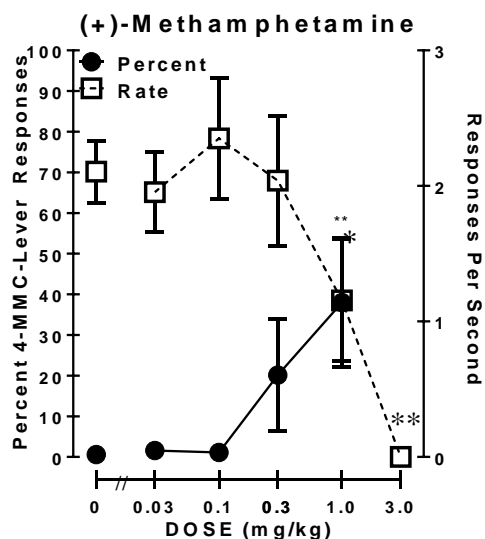


Figure C1. (+)-Methamphetamine dose-response curve in rats trained to discriminate 3.0 mg/kg mephedrone (n=8) from saline. Results are depicted as the mean (\pm SEM) percent 4-MMC-lever responses and the mean (\pm SEM) response rate in lever-presses per second. Symbols indicate response rates that significantly differed from rates observed during saline tests; * $p < .05$, ** $p < .01$, ## $p < .0001$.

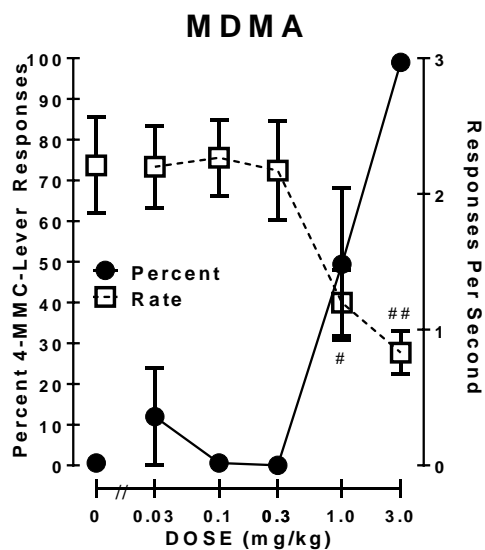


Figure C2. MDMA dose-response curve in rats trained to discriminate 3.0 mg/kg mephedrone (n=8) from saline. Results are depicted as the mean (\pm SEM) percent 4-MMC-lever responses and the mean (\pm SEM) response rate in lever-presses per second. Symbols indicate response rates that significantly differed from rates during saline tests; * $p < .05$, ** $p < .01$, ## $p < .0001$.