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SELF-ADMINISTRATION AND BEHAVIORAL ECONOMICS OF SECOND-GENERATION SYNTHETIC CATHINONES IN MALE RATS

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Abstract

Rationale—Synthetic cathinones have become increasingly available as drugs of abuse.

Distribution of these drugs is made possible by altering the chemical structures of prohibited cathinones and marketing them under misleading labels. Very little is known about the relative reinforcing effectiveness of new synthetic cathinones relative to known drugs of abuse.

Objective—We examined self-administration of three second-generation synthetic cathinones: alpha-pyrrolidinopentiophenone (alpha-PVP), 4-methyl-N-ethylcathinone (4-MEC), and 4-methyl-alpha-pyrrolidinopropiophenone (4-MePPP) relative to methamphetamine.

Method—Male, Sprague-Dawley rats, implanted with intravenous catheters, were trained to self-administer methamphetamine (0.05 mg/kg/injection) under a fixed-ratio schedule. Following training, various doses of methamphetamine (0.006-0.1 mg/kg/injection), alpha-PVP (0.0015-0.1 mg/kg/injection), 4-MEC (0.1-3.2 mg/kg/injection), or 4-MePPP (0.1-0.8 mg/kg/injection) were available for self-administration in separate groups, followed by a behavioral-economics evaluation of their reinforcing effectiveness.

Results—For all drugs, at least one dose functioned as a reinforcer. Alpha-PVP and 4-MePPP maintained the highest numbers of infusions per session and both were more effective reinforcers relative to methamphetamine. 4-MEC and methamphetamine were not significantly different in terms of infusions per session or reinforcing effectiveness.

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Author Note

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Conclusion—Emerging synthetic cathinones whose primary pharmacological mechanism is to block dopamine uptake but with little effects on monoamine release or serotonin uptake may have a greater degree of abuse potential compared with known abused stimulants.

Keywords

Methamphetamine; Synthetic cathinone; alpha-pyrrolidinopentiophenone (alpha-PVP); 4-methyl-N-ethylcathinone (4-MEC); 4-methyl- α -pyrrolidinopropiophenone (4-MePPP); Self-administration

Introduction

Synthetic cathinones (sometimes referred to as “bath salts”) are designed to mimic the effects of illicit stimulants such as cocaine, methamphetamine, and methylenedioxymethamphetamine (MDMA), but circumvent U.S. drug enforcement laws due to their novel chemical structures and marketing under misleading labels such as “bath salts” or “jewelry cleaner” (Drug Enforcement Administration; DEA 2011a; 2014a). Because of the strategy of synthesizing drugs “outside the law”, identification and control of synthetic cathinones is a particularly difficult challenge for both health care providers and law enforcement agencies. In 2011, the first-generation synthetic cathinones, methylenedioxypyrovalerone (MDPV), mephedrone, and methylone were placed under temporary schedule I status and since have been permanently scheduled (DEA 2011a; 2013a; 2013b). In 2014, ten second-generation synthetic cathinones were placed under temporary schedule I status, and their temporary scheduling recently was extended for an additional year (DEA 2014a; 2016). Despite their current illegal status, certain synthetic cathinones that have been scheduled are still bought through illicit channels and obtained at relatively cheap prices (Karila et al. 2015; DEA 2011b). The enduring popularity of synthetic cathinones suggests that at least some drugs within this class are more effective reinforcers than others, which presumably reflects unique neuropharmacological mechanisms of action (e.g., catecholamine blockers vs. empathogens).

Synthetic cathinones have been classified into three pharmacological subdivisions based on their particular effects at monoamine transporters (Iversen et al. 2014; Simmler et al. 2014). These pharmacological subdivisions parallel those for the illicit stimulants: 1) Blockers fall into the “cocaine-like” category as they block reuptake of monoamines but have no appreciable effect on monoamine release, 2) Releasers fall into the “methamphetamine-like” category, and in addition to functioning as blockers they also cause monoamine release and are catecholamine-preferring, and 3) Empathogens fall into the “MDMA-like” category and function as both blockers and releasers, but their releasing profiles for the catecholamines are less than or similar to the releasing profile for serotonin. There is relatively little basic research investigating synthetic cathinones as reinforcers, and we know less about how the pharmacological subdivisions of synthetic cathinones compare in terms of reinforcing strength. Cathinone was reported to be comparable to cocaine in reinforcing strength in monkeys using a drug vs. drug choice procedure (Woolverton and Johanson 1984). Furthermore, MDPV, but not mephedrone, was reported to be a stronger reinforcer than

methamphetamine in rats self-administering drugs under a progressive-ratio (PR) schedule of reinforcement (Aarde et al. 2013; Motbey et al. 2013; Watterson et al. 2014b).

In the present experiment, the abuse potential of three second-generation synthetic cathinones was assessed using drug self-administration procedures in rats: alpha-pyrrolidinopentiophenone (alpha-PVP), 4-methyl-*N*-ethylcathinone (4-MEC), and 4-methyl-alpha-pyrrolidinopropiophenone (4-MePPP). Alpha-PVP appears to have emerged as a replacement for MDPV (Baumann 2014; Paillet-Loilier et al. 2014; Marusich et al. 2014). 4-MEC is an analog that likely emerged as replacement for its parent compound, mephedrone (Baumann 2014; Paillet-Loilier et al. 2014; Saha et al. 2015). 4-MePPP has been described as an analog of mephedrone like 4-MEC (Saha et al. 2015) and as an analog of pyrovalerone, similar to MDPV and alpha-PVP (Gatch et al. 2015a). Alpha-PVP and 4-MePPP are blockers (but not releasers) at dopamine transporters (DAT), however in contrast with cocaine, alpha-PVP and 4-MePPP are more potent at blocking catecholamine transporters than the serotonin transporter (SERT; Marusich et al. 2014; Saha et al. 2015). 4-MEC's neurochemical profile is unique among stimulants in that it acts as an uptake inhibitor at DAT and a releaser at SERT (Iversen et al. 2013; Simmler et al. 2014; Saha et al. 2015).

Of the three second-generation cathinones in the present experiment, only alpha-PVP has been evaluated in a self-administration procedure, in which it functioned as a reinforcer under a simple fixed-ratio (FR) schedule and was similar to MDPV under a progressive-ratio (PR) schedule (Aarde et al. 2015). Drug-self administration is regarded as a "gold-standard" procedure among behavioral tests of abuse liability because most drugs of abuse are readily self-administered by non-human animals (e.g., Ator and Griffiths 2003; Horton et al. 2013; Huskinson et al. 2014). Self-administration is also a useful preclinical screen for informing the extent to which a drug is scheduled by regulatory agencies. Consistent with recommendations for abuse-potential testing of stimulant drugs (Huskinson et al. 2014), the goals of the current study were to 1) examine self-administration of alpha-PVP, 4-MEC, and 4-MePPP relative to the prototypical illicit stimulant, methamphetamine, 2) examine self-administration with several doses of each drug, and 3) determine the reinforcing effectiveness of each synthetic cathinone relative to methamphetamine using a behavioral-economics procedure.

Materials and Methods

All animal-use procedures were approved by the University of Mississippi Medical Center's Animal Care and Use Committee and were conducted in accordance with the National Research Council's Guide for Care and Use of Laboratory Animals (2011).

Subjects

A total of 26 male Sprague Dawley rats (Harlan Laboratories, New Jersey, USA) served as subjects. Each rat had served in the elevated-zero maze portion of a previous study (Gunter et al. 2016) in which each subject received a single injection of a test drug (benzodiazepine, neuroactive steroid, or combination) or its vehicle and were subsequently tested in the elevated-zero maze. They were approximately 70 days old and weighed between 260-300 g

at the start of the previous study and served in the present experiment within 14-60 days following the previous study. Six subjects were in the methamphetamine group, seven in the alpha-PVP group, seven in the 4-MEC group, and six in the 4-MePPP group. Rats were pair-housed and given free access to standard rat chow and water outside of experimental sessions. Housing and testing took place in a temperature-controlled vivarium that was maintained on a reverse 12-hr light-dark cycle with lights off at 8:00 am. Sessions took place during the dark phase.

Drugs

Final solutions for all drugs were prepared using 0.9% sterile saline, and doses are expressed as the salt form. Methamphetamine hydrochloride was purchased from Sigma (St. Louis, MO, USA). The synthetic cathinones (alpha-PVP, 4-MEC, and 4-MePPP) were synthesized as hydrochloride salts and were provided by Dr. Bruce Blough at the Research Triangle Institute (Research Triangle Park, NC, USA). During self-administration sessions, drugs were administered i.v. at a rate of 0.018 ml/s. Dose was adjusted by changing the drug concentration in the solution, based on each rat's body weight. Methohexital was administered i.v. to test catheter patency and was purchased from the University of Mississippi Medical Center pharmacy (Jackson, MS, USA).

Apparatus

Sessions were conducted in sixteen standard modular operant-conditioning chambers that were enclosed in ventilated, sound-attenuating chambers (Med-Associates, St. Albans, VT, USA). Each chamber was equipped with two retractable response levers and corresponding white stimulus light mounted above each lever. Drug infusions were delivered at a rate of 0.018 ml/s through 10 ml plastic syringes that were seated in infusion pumps (Razel Scientific, St. Albans, VT, USA). Polyethylene tubing connected the drug-filled syringe to a swivel located above the operant chamber from which a spring-arm leash was suspended. The terminal end of the leash consisted of a needle and a threaded tether that connected to the septum of a vascular access port (Instech Laboratories, Plymouth Meeting, PA, USA) that was implanted into the mid-scapular region of each subject (see Surgery). A desktop computer equipped with Med-Associates software (Med-PC for Windows; St. Albans, VT, USA) controlled all experimental conditions and recorded data.

Surgery

Subjects were implanted with intravenous catheters in the right jugular vein similar to that described previously (Townsend et al. 2015). Briefly, rats were anesthetized with inhaled isoflurane (2-3%), and the vein was isolated and catheterized with a polyethylene catheter (Instech Laboratories, Plymouth Meeting, PA, USA) that was routed subcutaneously to a vascular access port that was placed in the mid-scapular region. Subjects were administered carprofen (5 mg/kg injected s.c.) once pre-operatively and twice (24- and 48-h following surgery) post-operatively for pain. An antibiotic (gentamicin, 0.08 mg i.v.) was administered once daily for 7 days to prevent infection. Rats were allowed to recover for 5-7 days prior to experimental sessions. Catheters were flushed daily with 0.1 ml of heparinized saline (30 U/ml i.v.) post-session. Catheters were periodically tested for patency by injecting methohexital (5 mg/kg i.v.) followed by 0.1 ml of heparinized saline (30 U/ml i.v.).

Catheters were considered patent if ataxia was apparent within 5 s of injection. If a catheter was considered non-functional, a new catheter was implanted into the left jugular vein and the animal continued in the experiment. Catheter patency was also verified following the last behavioral economics session for each demand curve for each animal.

General Procedure

Sessions were conducted daily at approximately the same time each day. Sessions began in a dark chamber with the left and right levers extended into the chamber. Responses on the left lever resulted in a drug or saline injection (active lever), and responses on the right lever were recorded but had no other programmed consequences (inactive lever). When the response requirement on the left lever was completed (e.g., fixed-ratio 5 schedule of reinforcement; FR 5), both levers retracted, and the left cue light directly above the left lever was illuminated for the duration of the drug injection and a subsequent timeout that always summed to 10 s. Following each drug injection and timeout, the left cue light darkened and both levers were extended into the chamber, signaling drug availability. Sessions were 2 h, and no other restrictions were placed on the number of injections that could be delivered per session.

Initial Training—For methamphetamine and each synthetic cathinone tested, a separate group of rats was trained to self-administer 0.05 mg/kg/injection methamphetamine under a FR 1 schedule of reinforcement until 10 or more injections were obtained during a session, and 20% or fewer of total lever presses were on the inactive lever for two consecutive sessions. Following these criteria, the response requirement was raised to an FR 5 with the same dose of methamphetamine available for self-administration. Methamphetamine was available under these conditions for a minimum of 5 sessions and until the following stability criteria were met in the final 3 sessions: 1) a mean of 10 or more injections per session, 2) the number of infusions was within 20% of the 3-session mean, 3) 20% or fewer of total lever presses were on the inactive lever, and 4) no upward or downward trends. Because of the relatively short catheter life in rats, and to increase the likelihood that all subjects would complete all conditions with functional catheters, if stability criteria for a condition were not met within 10 days, the condition was ended and a new condition began.

Dose-response determinations—Following acquisition of self-administration with 0.05 mg/kg/injection of methamphetamine, subjects were assigned to different drug groups to obtain dose-response functions for each drug: methamphetamine (n=6), alpha-PVP (n=7), 4-MEC (n=7), or 4-MePPP (n=6). Depending on the drug group, saline and at least three doses of methamphetamine (0.006-0.1 mg/kg/injection), alpha-PVP (0.0015-0.1 mg/kg/injection), 4-MEC (0.1-3.2 mg/kg/injection), or 4-MePPP (0.1-0.8 mg/kg/injection) were made available under a FR 5 schedule until responding was considered stable. For doses under which an average of 10 or more infusions were delivered per session, the stability criteria were the same as described above for methamphetamine training at FR 5 with the exception that a minimum of 3 sessions (rather than 5) were required. For saline and doses under which an average of less than 10 infusions were delivered per session, responding was considered stable when the following criteria were met: 1) a minimum of 3 sessions and 2) no trends in the total number of infusions per session across the final 3 sessions. If stability

criteria were not met within 10 sessions during dose-response determinations, the condition was ended and a new condition began. Conditions in which stability criteria were not met is indicated for individual subjects by an asterisk in Table 1.

Doses were initially selected based on the available literature and on preliminary data with the synthetic cathinones. Doses were adjusted on an individual-subject basis, and in a counterbalanced order, in an attempt to obtain complete dose-response functions for individual subjects (i.e., an inverted U-shaped curve) with at least one dose on the ascending limb, a peak dose, and one dose on the descending limb. Doses experienced by each subject in each group are shown in Table 1. In two instances with 4-MePPP (subjects 43 and 44), an inverted U was not obtained for individual subjects. For these subjects, an average of 197 and 210 injections were delivered at the 0.1 mg/kg/injection dose, and a smaller dose was not determined because more than 300 injections were delivered per session at 0.05 mg/kg/injection. In these instances, the syringes often ran out of solution before they could be replaced. For these subjects, a portion of the descending limb was obtained.

Behavioral economics demand curves—Following dose-response determinations under an FR 5 schedule, a behavioral-economics procedure was used to determine the reinforcing effectiveness of each of the drugs. One subject in the alpha-PVP group, one subject in the 4-MEC group, and two subjects in the 4-MePPP group did not complete demand curves because of non-functional catheters. The rate of attrition resulting from nonfunctional catheters was greater for 4-MePPP than the other drugs tested. For this reason, demand was completed in four animals in the 4-MePPP group. For all drugs, the dose(s) used for completion of demand curves was selected on an individual-subject basis because there was between-subject variability in dose-response determinations (see Table 1 for doses used for individual subjects). Specifically, for each demand curve, the first dose on the descending limb of the individual subject's dose-response function was used (see also Negus et al. 2007, for a similar use of this approach). For one rat (subject 44) that completed 4-MePPP demand, the ascending limb and peak dose was not obtained (see description above for dose-response determinations). In this instance, a dose on the descending limb was selected by choosing the dose that resulted in similar levels of self-administration as the doses used with other subjects in the 4-MePPP group. For determination of each demand curve, the selected dose was available under a FR 5 schedule for consecutive sessions until responding was considered stable according to the same stability criteria described above for dose-response determinations. Once responding was stable, the FR value was raised daily, until zero infusions were delivered in a single session in the following order: 8, 12, 18, 27, 41, 62, 93, 140, 210, 315 (i.e., each value was 1.5 times the preceding response requirement).

Data Analysis

For methamphetamine training and dose-response determinations, the mean number of infusions per session for each subject during the 3 stable sessions of a condition was used in all analyses when stability criteria were met within 10 sessions. In the small number of instances when stability criteria were not met within 10 sessions, the mean of the last 5 sessions was used for data analysis to ensure appropriate representation of the variability in number of injections obtained in these cases. Table 1 indicates with an asterisk all instances

in which stability criteria were not met. To demonstrate that the training dose of methamphetamine functioned as a reinforcer for each group, mean infusions per session was compared to saline using paired t-tests. To ensure that each drug group self-administered similar amounts of methamphetamine during training, mean infusions of methamphetamine per session was compared across drug groups using a one-way analysis of variance (ANOVA) and Bonferroni t-tests for multiple comparisons.

For dose-response determinations, repeated-measures analysis of variance (ANOVA) followed by Dunnett's multiple-comparisons tests were used to compare the mean number of infusions per session for each dose with saline. For one subject, alpha-PVP was self-administered at smaller doses relative to other subjects in that group (see **Table 1**). This subject was not included in the repeated-measures ANOVA because only one dose was within the range self-administered by all 7 subjects. For the remaining 6 subjects in the alpha-PVP group and for all subjects in the other drug groups, only doses that were experienced by all subjects were used in the repeated-measures ANOVA. These doses were 0.012, 0.025, and 0.05 mg/kg/injection for methamphetamine, 0.012 and 0.025 mg/kg/injection for alpha-PVP, 0.4 and 0.8 for 4-MEC, and 0.1, 0.2, and 0.4 mg/kg/injection for 4-MePPP.

For demand curves, log consumption (mean #injections/session) was plotted as a function of price (i.e., the FR value or number of lever presses required for a single drug injection). The maximum FR value used for each drug group was the largest FR that resulted in at least one drug injection for at least one subject within a drug group. This was done to maintain a mean number of injections above zero as zeros cannot be plotted on a log scale. The maximum FR was 93 for methamphetamine, 210 for alpha-PVP, 93 for 4-MEC, and 140 for 4-MePPP. For subjects within a drug group that met the zero-injection criterion at a smaller FR value than was used in the equation, a 0 was entered for subsequent FR values. This was done to avoid inflated means at the larger FR values of each demand curve. For example, the maximum FR value in the alpha-PVP group was 210; if a subject in that drug group received 0 injections at FR 93, then a 0 was entered for FR 93, 140, and 210. The elasticity of the resulting demand curves was calculated using a custom-designed GraphPad Prism 5.0 template (freely available from the Institutes for Behavior Resources, www.ibrinc.org) and the group means were fit with the Exponential Model of Demand introduced by Hursh and Silberberg (2008) with k (a scaling parameter) set at 4. Demand elasticity (α) was compared using an extra sum-of-squares F test to determine whether the demand curves for methamphetamine and each synthetic cathinone were better described by a single curve fit to both data sets or by a separate fit for each data set using the Exponential Model of Demand. In addition, 95% confidence intervals for α were calculated from the average demand curves for each drug.

Results

All subjects self-administered 0.05 mg/kg/injection of methamphetamine during training under an FR 5 schedule at levels significantly greater than saline [methamphetamine group: $t(5)=5.8$, $p<.01$; alpha-PVP group: $t(6)=6.3$, $p<.001$; 4-MEC group: $t(6)=8.6$, $p<.001$; 4-MePPP group: $t(5)=7.6$, $p<.001$], and there were no significant differences between drug groups in the mean number of methamphetamine injections per session during training.

Figure 1 shows the mean number of infusions per session obtained during the stable sessions (or the last 5 sessions when stability criteria were not met) for saline availability and each dose of methamphetamine, alpha-PVP, 4-MEC, and 4-MePPP for each group. All four drugs maintained average numbers of infusions/session above the levels maintained during sessions with saline available for at least one dose, indicating that each drug functioned as a reinforcer. The doses that maintained self-administration significantly above saline levels for each drug were 0.025 and 0.05 mg/kg/injection of methamphetamine, 0.012 and 0.025 mg/kg/injection of alpha-PVP, 0.8 mg/kg/injection of 4-MEC, and 0.2 and 0.1 mg/kg/injection of 4-MePPP ($p < .05$). An inverted U-shaped dose-effect function was obtained for each drug with the peak of the curve occurring at a 0.025 mg/kg/injection for methamphetamine [$M=0.021$, 95% CI (0.014,0.028)], 0.012 mg/kg/injection for alpha-PVP [mean=0.012, 95% CI (0.006,0.018)], 0.8 mg/kg/injection for 4-MEC [mean=0.74, 95% CI (0.35,1.14)], and 0.2 mg/kg/injection for 4-MePPP [mean=0.17, 95% CI (0.11,0.22)], indicating that the rank order of potency as reinforcers in this procedure was alpha-PVP = methamphetamine > 4-MePPP > 4-MEC.

At the group level, there were significant differences in the maximum amount of behavior maintained by methamphetamine and each synthetic cathinone. At the peak doses, alpha-PVP (0.012 mg/kg/injection) [mean = 98.6, 95% CI (77.7,119.5)] and 4-MePPP (0.2 mg/kg/injection) [mean=91.8, 95% CI (57.1,126.4)] were self-administered at higher rates (injections/session) relative to methamphetamine (0.025 mg/kg/injection) [mean=55.1, 95% CI (32.3,77.9); $p < .05$]. However, the amount of 4-MEC injections delivered at the peak dose (0.8 mg/kg/injection) [mean=26.8, 95% CI (12.7,40.9)] was not significantly different from methamphetamine ($p=.10$). Individual differences in the peak dose and maximal behavioral output were observed, though the overall trend observed at the group level was apparent at the individual-subject level. Table 1 shows mean injections/session and SEM for individual subjects in each group at each dose experienced.

Figure 3 shows demand curves plotted as aggregated log consumption as a function of log price for methamphetamine (the same demand function is shown in each panel for comparison), alpha-PVP (Figure 3A), 4-MEC (Figure 3B), and 4-MePPP (Figure 3C). The α term (i.e., a free parameter that reflects demand elasticity), Q_0 (i.e., consumption at 0 price), and R^2 values for methamphetamine and each synthetic cathinone are shown in each legend. Alpha-PVP [95% CI of α (0.00002,0.000046)] and 4-MePPP [95% CI of α (0.000027,0.000048)] were less elastic (i.e., greater demand) than methamphetamine [95% CI of α (0.000056,0.0001)], and this was indicated by a smaller α values. The alpha-PVP and methamphetamine curves were best described by different alpha values [$F(1,14)=16.0$, $p<.01$], and similarly, 4-MePPP and methamphetamine curves were best described by different alpha values [$F(1,13)=19.65$, $p<.001$]. Conversely, 4-MEC [95% CI of α (0.000057,0.00019)] and methamphetamine were not different, indicating similar elasticity of demand [$F(1,12)=2.8$, $p=.12$].

Discussion

The abuse potential of alpha-PVP, 4-MEC, and 4-MePPP, three second-generation synthetic cathinones temporarily placed under schedule I status, was assessed using two self-

administration procedures (i.e., a FR 5 schedule and behavioral economics). Self-administration under the FR arrangement was examined at multiple doses of each synthetic cathinone to obtain a broad range of doses and was compared to a prototypical illicit stimulant, methamphetamine. Alpha-PVP, 4-MEC, and 4-MePPP all functioned as reinforcers, suggesting that, at least at a relatively low cost (i.e., FR 5), these drugs may have abuse liability. Recently, Aarde et al. (2015) examined alpha-PVP self-administration by rats under a FR 1 schedule of reinforcement in 1-hr sessions. While Aarde and colleagues did not obtain an ascending limb under their FR procedure, the descending limbs of alpha-PVP in the current paper and in their experiment are nearly identical. To our knowledge, the current experiment is the first laboratory demonstration of self-administration of 4-MEC and 4-MePPP.

Alpha-PVP and 4-MePPP maintained more behavior (injections/session) than methamphetamine at the peak dose for each drug, and 4-MEC was similar to methamphetamine. It is possible that the high level of behavioral output is an indicator that alpha-PVP and 4-MePPP are more effective reinforcers relative to methamphetamine. However, because rates of responding under FR schedules of reinforcement do not necessarily reflect a drug's relative effectiveness as a reinforcer, we used a behavioral-economics procedure to more selectively assess the reinforcing effectiveness of each synthetic cathinone relative to methamphetamine. A unique feature of the behavioral-economics approach under FR arrangements is that the elasticity of the demand function is thought to be independent of dose, allowing comparison between different types of drugs at single, albeit functionally similar, doses (Bickel et al. 1990; Hursh and Winger 1995). Despite the argument that demand functions are dose independent, we selected a functionally-matched dose for each subject within a drug group (i.e., the first dose on the descending limb of each subject's dose-response determination) when possible. Using this approach, both alpha-PVP and 4-MePPP were more effective reinforcers than methamphetamine, and 4-MEC was similar in reinforcing effectiveness relative to methamphetamine, suggesting that alpha-PVP and 4-MePPP may have a greater potential for abuse than methamphetamine. Our results with alpha-PVP are indirectly supported by Aarde et al. (2015) who found alpha-PVP to be equal in reinforcing effectiveness relative to MDPV using a PR procedure, and Aarde et al. (2013) who found MDPV to be greater in reinforcing effectiveness relative to methamphetamine. Thus, by inference, alpha-PVP would also be expected to be greater in reinforcing effectiveness relative to methamphetamine, and results from the current experiment provide direct evidence that this is the case.

It was hypothesized that differences in the neuropharmacological profiles of the synthetic cathinones would be associated with differences in their relative reinforcing effectiveness. Alpha-PVP and 4-MePPP were more effective reinforcers than methamphetamine under the current testing conditions as evidenced by their relative inelasticity of demand. The fact that both of these drugs are catecholamine-preferring blockers with negligible releasing effects suggests that stimulants that selectively block catecholamine transporters will have significant abuse liability. In neurochemical assays, 4-MePPP increased extracellular DA but not 5-HT, effects similar to alpha-PVP and MDPV, which could contribute to the similarity in reinforcing effectiveness found in the current study. However, consistent with its short

duration of effect on stimulating locomotor activity, 4-MePPP's ability to increase extracellular DA is short lived (Saha et al. 2015). Other behavioral studies with alpha-PVP and 4-MePPP have identified some differences between these drugs. For example, alpha-PVP and 4-MePPP fully substituted for methamphetamine in a drug-discrimination procedure (Gatch et al. 2015a; Naylor et al. 2015, Smith et al. 2016), and while alpha-PVP fully substituted for cocaine, 4-MePPP narrowly missed the commonly used 80% criterion for full substitution, reaching 75% cocaine-lever responding (Gatch et al. 2015a). In a test of drug reward, Gatch et al. (2015a) found that alpha-PVP resulted in conditioned place preferences in rats while 4-MePPP did not. Based on these findings, the authors concluded that 4-MePPP would less likely than alpha-PVP to emerge as a novel stimulant of abuse, a conclusion not supported by the current findings.

One explanation for discrepant results between self-administration and conditioned place preference is that the latter requires a single injection of drug per daily conditioning session. Given that 4-MePPP has a relatively low potency and short duration of effect, a single administration may not have been sufficient to detect reinforcement-related effects. Under conditions of self-administration, rats in the current report were able to adjust response rates to control drug intake, and they did so via a route of administration (i.e., intravenous) that generally increases the potency of psychoactive drugs, conditions that should increase the likelihood of detecting effects from short-acting drugs with low potency. Thus, 4-MePPP's apparent short duration of action combined with its relatively low potency in behavioral and neurochemical assays suggests that users would need to obtain much more drug for shorter-lived effects, and this could contribute to the lack of appearance of 4-MePPP in confiscated samples (DEA, 2014a; 2014b).

The rate of attrition resulting from nonfunctional catheters was greater for 4-MePPP than the other drugs tested. For this reason, demand was completed in four animals in the 4-MePPP group. It is possible that more subjects would have resulted in a different outcome, and some caution should be taken in interpreting the results obtained with 4-MePPP. However, it is also important to note that the current experiment used a single-subject design within each drug group such that doses tested in individual subjects were functionally relevant and were based on individual-subject behavior. The use of a single-subject design and functionally relevant doses (i.e., doses on the descending limb) likely reduced variability that might have been observed if a single dose had been selected and tested in all subjects.

The finding that 4-MEC was self-administered but was no different than methamphetamine in reinforcing effectiveness is important for a number of reasons. First, the results demonstrate that differences in reinforcing potency between drugs do not necessarily predict relative reinforcing effectiveness in the behavioral-economic design. 4-MEC was self-administered at larger doses (i.e., was much less potent) than methamphetamine, but both drugs were comparable in elasticity of demand. Second, 4-MEC produces effects similar to alpha-PVP and 4-MePPP as a blocker at catecholamine transporters and effects similar to alpha-PVP and methamphetamine in its ability to reduce intracranial self-stimulation thresholds (Watterson et al. 2014a). Like alpha-PVP, 4-MEC substituted for cocaine and methamphetamine, but only at relatively high doses (Gatch et al. 2015b), and 4-MEC did not substitute for methamphetamine at relatively smaller, yet behaviorally active doses (Naylor

et al. 2015). Unlike alpha-PVP and 4-MePPP, 4-MEC did not produce a greater reinforcing effect than methamphetamine. Key to this finding may be the fact that 4-MEC is atypical among the synthetic cathinones in that it functions as a blocker of catecholamine transport but also as a releaser at the serotonin transporter (Iversen et al. 2013; Simmler et al. 2014; Saha et al. 2015). Drugs that have prominent serotonin-releasing effects (e.g., MDMA) generally function as weaker reinforcers than illicit stimulants like cocaine and methamphetamine (e.g., Lile et al. 2005; Wang and Woolverton 2007). Thus, it may be that 4-MEC's apparent reinforcing effect is driven by catecholamine blockade but tempered by serotonin release.

The abuse rates of novel synthetic cathinones will likely peak and wane as regulatory measures evolve to control the appearance of each emerging layer of drugs. However, within this "pipeline" of untested stimulants, drugs with novel pharmacological profiles will continue to appear, thus necessitating the need to better understand the relationship between the abuse potential of stimulants and the varied configurations of their monoamine pharmacology. Developing a comprehensive understanding of the pharmacological variables that modulate the reinforcing effectiveness of synthetic cathinones and other novel stimulants may also be useful for prioritizing the scheduling of compounds according to functional profile rather than chemical structure.

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Fig. 1.

Mean number of injections per session during self-administration of methamphetamine (Meth; $n=6$) and the 2nd generation synthetic cathinones alpha-PVP ($n=7$), 4-MEC ($n=7$), and 4-MePPP ($n=6$) in rats. Numbers in parenthesis indicate the number of subjects that experienced a particular dose not experienced by all subjects. Error bars are one standard error of the mean (SEM) and S = saline. Asterisks indicate significant differences relative to saline, $p < 0.05$.

Fig. 2.

Mean demand curves plotted as log consumption (mean #injections/session) as a function of log price (FR value) for methamphetamine (closed circles; same curve in all three panels), alpha-PVP (open circles, panel A), 4-MEC (triangles, panel B), and 4-MePPP (squares, Panel C). The α , Q_0 , and R^2 values resulting from each curve fit are shown for each drug group in each legend, and error bars represent 95% confidence intervals.

Table 1

Individual-subject data for dose-response determinations for each of the four drug groups. The doses tested are shown above each group of subjects and mean injections/session (SEM) is shown for each dose. The boldface font indicates the dose that was used for subjects in the demand portion of the experiment.

Subject	Saline	Methamphetamine (mg/kg/injection)				
		0.006	0.012	0.025	0.05	0.1
S18	6.3 (1.2)	-	15.0 (1.7)	38.7 (2.6)	30.3 (0.7)	25.0(0.6)
S19	13.0 (1.5)	-	13.0 (1.8) *	29.7 (1.8)	23.2 (1.0)	14.7(0.3)
S22	14.8 (1.7) *	67.7 (6.0)	89.0 (2.6)	68.3 (3.2)	37.6 (0.8) *	-
S27	8.3 (0.9)	-	18.8 (3.6) *	76.0 (2.9)	46.3 (1.9)	-
S31	4.3 (1.3)	-	3.7 (0.3)	79.0 (2.1)	45.7 (2.8)	25.0 (1.2)
S41	9.0 (1.2)	25.3 (1.3)	73.3 (4.9)	39.0 (4.0) *	31.0 (1.0)	-

Subject	Saline	Alpha-PVP (mg/kg/injection)						
		0.0015	0.003	0.006	0.012	0.025	0.05	0.1
S02	8.0 (0.6)	-	9.0 (2.3)	148.3 (13.9)	96.7 (4.5)	53.7 (3.2)	35.7 (0.9)	-
S05	8.7 (0.3)	-	-	105.0 (23.4) *	131.7 (5.2)	75.7 (0.7)	54.3 (1.9)	-
S07	7.3 (0.3)	-	-	4.67 (0.3)	80.3 (1.7)	57.3 (3.3)	56.7 (5.8)	-
S08	9.7 (2.9)	-	-	-	85.0 (4.4)	141.3 (4.1)	51.3 (2.2)	25.3 (1.2)
S09	8.7 (0.9)	-	-	13.7 (1.5)	129.7 (1.8)	78.7 (0.7)	36.0 (0.6)	-
S10	8.3 (0.3)	-	-	37.3 (1.3)	80.7 (0.9)	57.3 (1.3)	-	-
S14	15.6 (1.3) *	30.0 (2.0)	162.4 (21.0) *	142.0 (6.6)	86.0 (2.1)	-	-	-

Subject	Saline	4-MEC (mg/kg/injection)					
		0.1	0.2	0.4	0.8	1.6	3.2
S13	4.7 (2.0)	-	5.7 (2.4)	12.3 (1.2)	6.7 (1.5)	8.0 (0.0)	7.7 (0.3)
S16	9.3 (1.2)	-	-	12.3 (0.3)	41.7 (1.2)	27.3 (1.8)	17.0 (0.0)
S17	9.7 (0.9)	-	-	8.3 (2.9)	49.0 (2.5)	32.3 (1.8)	17.3 (0.7)
S46	9.3 (0.3)	-	-	4.3 (1.2)	23.0 (2.1)	31.0 (1.5)	25.7 (0.3)
S48	4.0 (0.6)	-	-	9.0 (1.0)	26.3 (2.3)	21.8 (4.4) *	-
S51	8.0 (3.0)	-	1.0 (0.0)	20.7 (1.7)	11.0 (0.0)	-	-
S54	5.3 (2.0)	9.0 (3.1)	45.7 (4.7)	34.7 (2.7)	29.7 (0.9)	-	-

4-MePPP	Saline	4-MePPP (mg/kg/injection)			
		0.1	0.2	0.4	0.8
S28	9.3 (0.7)	6.0 (1.5)	126.0 (1.2)	68.7 (2.2)	-
S29	6.0 (1.5)	54.6 (10.2) *	118.3 (4.3)	66.7 (3.5)	34.0 (1.2)
S42	4.3 (2.2)	19.0 (3.1) *	43.7 (3.3)	36.0 (1.7)	-

S43	7.7 (1.3)	197.7 (2.9)	59.3 (5.7)	63.3 (1.3)	-
S44	6.7 (0.3)	210.0 (14.0)	98.3 (4.3)	92.0 (5.5)	-
S53	7.3 (0.7)	15.0 (2.3) *	105.0 (3.1)	80.0 (6.0)	-

* data points that included last 5 session of a condition when stability criteria were not met within 10 days