

In vivo potency and efficacy of the novel cathinone α -pyrrolidinopentiophenone and 3,4-methylenedioxypyrovalerone: self-administration and locomotor stimulation in male rats

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Abstract

Rationale Numerous substituted cathinone drugs have appeared in recreational use. This variety is often a response to legal actions; the scheduling of 3,4-methylenedioxypyrovalerone (MDPV; “bath salts”) in the USA was followed by the appearance of the closely related drug α -pyrrolidinopentiophenone (alpha-PVP; “flakka”).

Objectives This study aimed to directly compare the efficacy and potency of alpha-PVP with that of MDPV.

Methods Groups of male Wistar rats were trained in the intravenous self-administration (IVSA) alpha-PVP or MDPV under a fixed-ratio 1 schedule of reinforcement. An additional group was examined for locomotor and body temperature responses to noncontingent administration of MDPV or alpha-PVP (1.0, 5.6, and 10.0 mg/kg, i.p.).

Results Acquisition of alpha-PVP (0.1 mg/kg/infusion) IVSA resulted in low, yet consistent drug intake and excellent discrimination for the drug-paired lever. Dose substitution (0.05–0.25 mg/kg/infusion) under a fixed-ratio 1 schedule confirmed potency was similar to MDPV in prior studies. In direct comparison to MDPV (0.05 mg/kg/infusion), rats trained on alpha-PVP (0.05 mg/kg/infusion) responded for more infusions but demonstrated similar drug-lever discrimination by the end of acquisition. However, the dose–response (0.018–0.56 mg/kg/infusion) functions of these drugs under a

progressive-ratio schedule of reinforcement reflected identical efficacy and potency. Peak locomotor responses to MDPV or alpha-PVP were observed after the 1.0 mg/kg, i.p. dose and lasted ~2 h. Modest body temperature decreases were of similar magnitude (~0.75 °C) for each compound.

Conclusions The potency and efficacy of MDPV and alpha-PVP were very similar across multiple assays, predicting that the abuse liability of alpha-PVP will be significant and similar to that of MDPV.

Keywords Stimulants · Substance abuse · Bath salts · Self-administration · Cathinone · Reward

Introduction

A diversity of substituted cathinone stimulants have appeared on the recreational use markets worldwide over the past half-decade. Some of this variety is apparently being driven by legal control actions for one specific compound resulting in the emergence of a closely related replacement. The US Drug Enforcement Administration (DEA) made a temporary scheduling action for 3,4-methylenedioxypyrovalerone (MDPV; aka “bathsalts”) in 2011 (DEA 2011), and Congress permanently scheduled it in 2012. This was followed by the appearance of the closely related α -pyrrolidinopentiophenone (alpha-PVP; aka “gravel” or “flakka”) in the recreational market. Alpha-PVP has already been associated with human fatalities (Nagai et al. 2014; Wurita et al. 2014), and, while the US DEA placed alpha-PVP on the Schedule I in 2014 (Drug Enforcement Administration 2014) in a temporary scheduling action, there are few data attesting to the abuse liability of alpha-PVP. The direct determination of abuse liability using validated animal models is critical for understanding likely risks of specific substituted cathinones because inferences

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based on similar chemical structure alone can be misleading. For example, the neuropharmacological, locomotor stimulant and thermoregulatory effects of 4-methylmethcathinone/mephedrone (Baumann et al. 2012; Huang et al. 2012; Miller et al. 2013a; Wright et al. 2012) are similar to those of 3,4-methylenedioxymethamphetamine (MDMA). Nevertheless, this compound is considerably more effective than MDMA (De La Garza et al. 2007; Feduccia et al. 2010; Schenk et al. 2007) in rat models of intravenous self-administration (Aarde et al. 2013a; Hadlock et al. 2011; Motbey et al. 2013). The 3,4-methylenedioxy motif that distinguishes MDMA from methamphetamine—a readily self-administered amphetamine-class stimulant—also distinguishes MDPV from alpha-PVP (Fig. 1). MDMA is only poorly and inconsistently self-administered by rats as compared with methamphetamine (De La Garza et al. 2007; Feduccia et al. 2010; Schenk et al. 2007). It remains to be determined whether or not this particular molecular change to the readily self-administered MDPV (Aarde et al. 2013b) to generate alpha-PVP yields the same distinction in the reinforcer potency and efficacy with these restricted transporter blockers, as it does for the amphetamines which have monoamine transporter substrate and monoamine releaser properties.

Alpha-PVP has been recently shown to increase locomotor behavior in mice (Kaizaki et al. 2014; Marusich et al. 2014) with a potency that is less than that of MDPV. It was also slightly less potent at inhibiting the dopamine transporter compared with MDPV (Marusich et al. 2014), which was consistent with the mouse locomotor data, and less effective in reducing intracranial self-stimulation reward thresholds (Watterson et al. 2014a, b). This predicts that alpha-PVP would be *less* potent as a reinforcer in supporting intravenous self-administration behavior in rat models (Wee et al. 2006). Nevertheless, higher selectivity of alpha-PVP for the dopamine transporter (DAT) over the serotonin transporter (SERT) compared with MDPV suggests that alpha-PVP might be even *more* potent, since DAT/SERT ratio is more predictive of reinforcer potency than DAT affinity alone (Roberts et al. 1999; Wee et al. 2005).

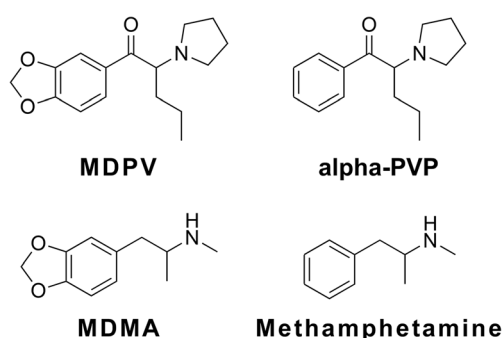


Fig. 1 Chemical structures of cathinone stimulants α -pyrrolidinopentiophenone (*alpha*-PVP) and 3,4-methylenedioxypyrovalerone (MDPV) are compared with the amphetamines methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA)

This study was undertaken to directly compare the potency and efficacy of alpha-PVP with that of MDPV in established rat models of stimulant abuse liability, thermoregulation, and activity. These approaches therefore permit additional inferences to be drawn to related investigations of other psychomotor stimulants. Studies in this study were conducted to compare drug intake across the acquisition of intravenous self-administration and then during subsequent dose substitution under fixed- and progressive-ratio schedules of reinforcement. The stimulants were also compared for their potential to alter body temperature and locomotor activity using a minimally invasive radiotelemetry system, which has the benefit of minimizing potential handling artifacts. Locomotor behavior is a frequently used behavioral assessment of psychomotor stimulants, and the potential to disrupt body temperature is relevant both to human medical emergency—including MDPV case reports (Borek and Holstege 2012; Froberg et al. 2015; Kesha et al. 2013)—and lasting neurotoxicity in high dose rodent models. Thus, the study extends the comparison of these drugs across additional major endpoints of interest.

Materials and methods

Subjects

Male Wistar rats (Charles River, New York) were used for these investigations. Subjects were housed in groups of two to three in humidity and temperature-controlled (23 ± 1 °C) vivaria on 12:12 h light/dark cycles. Rats entered the laboratory at 10–13 weeks of age and weighed 350–400 g at the start of the study. All rats had ad libitum access to food and water in their home cages. All procedures were conducted 4–5 days/week in the dark cycle starting 0–3 h after lights out. Studies were conducted under protocols approved by the Institutional Care and Use Committees of The Scripps Research Institute.

Drugs

The racemic 3,4-methylenedioxypyrovalerone HCl used for this study was obtained from Fox Chase Chemical Diversity Center (Doylestown, PA, USA) from synthetic routes designed by author TJD. Racemic α -pyrrolidinopentiophenone HCl was obtained from Cayman Chemical Company (Ann Arbor, MI, USA). All MDPV and alpha-PVP doses are expressed as the salt and drugs were dissolved in physiological saline for intravenous infusions or intraperitoneal injections. Cefazolin (Hikma Farmaceutica, Portugal), Flunixin (Bimeda USA, Oakbrook Terrace, IL, USA) and Brevital (Methohexital Sodium; JHP Pharmaceuticals, Rochester, MI, USA) were used for surgical and catheter care as described below.

Intravenous catheterization

Rats (MDPV, $N=18$; alpha-PVP, $N=27$) 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, b). Briefly, the catheters consisted of a 14-cm length of polyurethane-based tubing (Micro-Renathane®, Braintree Scientific, Inc., Braintree MA, USA) fitted to a guide cannula (Plastics One, Roanoke, VA, USA) 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 rat'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 (3 M™ Vetbond™ Tissue Adhesive; 1469SB).

A minimum of 4 days was allowed for surgical recovery prior to starting an experiment. For the first 3 days of the recovery period, the antibiotic Cefazolin (Hikma Farmaceutica, Portugal; 0.4 mg/kg, i.m. in sterile water day 1, s.c. days 2–3) and the analgesic flunixin (FlunixinJect, Bimeda USA, Oakbrook Terrace, IL, USA; 2.5 mg/kg, s.c. in saline) were administered daily. During recovery, as well as 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 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 in saline; JHP Pharmaceuticals, Rochester, MI, USA). Rats with patent catheters exhibit prominent signs of anesthesia (pronounced loss of muscle tone) within 3 s after infusion. Rats that failed to display these signs were considered to have faulty catheters and were discontinued from the study. Rats that failed to complete a given phase of the study with patent catheters were excluded from analysis.

Radiotelemetry implantation

Sterile radiotelemetry transmitters (Data Sciences International; TA-F40) were implanted in the abdominal cavities of a group of rats ($N=8$) thru an incision along the abdominal midline posterior to the xyphoid space as previously reported (Miller et al. 2013a; Wright et al. 2012). Absorbable sutures were used to close the abdominal muscle incision, and the skin incision was closed with the aforementioned tissue adhesive. Postoperative care and recovery time were the same as that for i.v. catheterization.

Self-administration procedure

Drug self-administration was conducted in 1 h (3 h for progressive ratio, see below) sessions using operant conditioning chambers [Med Associates; interior dimensions (30.5 cm $L \times$ 24.1 cm $W \times$ 21.0 cm H)] 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 fitting on a rat's back was connected to polyethylene tubing contained inside a protective spring (the “tether”) suspended into the operant chamber from a liquid swivel attached to a balance arm. The tether was filled with the drug solution prior to connection to the catheter, and then, the catheter tubing volume was filled after connection. Each operant session started with the extension of two retractable levers into the chamber. Following each completion of the response requirement (response ratio) on the drug-associated lever (right for all rats), a white stimulus light (located above the drug-associated lever) signaled delivery of the reinforcer and remained on during a 20-s postinfusion timeout, during which responses were recorded but had no other scheduled consequences. Responses on the alternate lever were recorded but led to no scheduled consequences. Rats were primed with a single infusion per session if none were observed within 30 min of the start of the session. Drug infusions (0.1 ml/infusion, 4 s/infusion) were delivered via syringe pump. Separate groups of rats were trained to self-administer MDPV ($N=18$; 0.05 mg/kg/infusion) or alpha-PVP ($N=9$; 0.1 mg/kg/infusion, $N=18$; 0.05 mg/kg/infusion). The response requirement was fixed-ratio 1, and session duration was 1 h during the acquisition phase, which was schedule a priori for 20 sessions.

Fixed-ratio 1 dose–response testing The rats trained on alpha-PVP (0.1 mg/kg/infusion) were subjected to randomized dose-substitution conditions in which the per-infusion dose differed (0.0, 0.025, 0.05, 0.1, 0.25 mg/kg/infusion) on alternate (daily) sessions (1 h duration) starting after the acquisition interval. The dose orders were counterbalanced by a Latin Square design, and each rat received two sequential blocks of dose series (an individual's data were averaged across block for analysis).

Progressive-ratio, dose–response testing Following the fixed-ratio (FR) series, the rats were subjected to randomized dose substitution under a progressive-ratio (PR) schedule of reinforcement. 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 = $5e^{(\text{injection number} \times j)} - 5$ (Richardson and Roberts 1996). The value of “ j ” for this study was 0.4 and was based upon a prior study with MDPV (Aarde et al. 2013b) so as to

observe a “breakpoint” in ~3 h at 0.10 mg/kg/infusion. The dose orders were counterbalanced by a Latin Square design, and each rat received two sequential blocks of dose series. Thus, an individual experienced each dose condition once within each block, and the data were averaged across the two determinations of each dose for analysis. Sessions ended after 3 h or the observation of an interinfusion interval of 1 h.

Telemetry procedure

Rats were assessed for 30 min prior to injection to ensure stable temperature and activity baseline, subsequent to any effects of handling or of moving to the procedure room. Rats were evaluated in a normal housing cage, with bedding material, placed on top of the telemetry receiver plate. Seven treatment conditions (Veh; 1, 5.6, and 10 mg/kg of alpha-PVP and MDPV) were counterbalanced by a Latin Square design. Drugs were injected i.p. (1.0 ml/kg volume) with a 3–4-day interval between sessions. The procedure room was at 21 °C (± 1 °C) for telemetry studies, and all rats were assessed in single-housing conditions. The data (activity rate, core body temperature in degrees Celsius) were recorded at 5-min intervals and were represented as 15-min averages for analysis and presentation.

Data analysis

Data were analyzed with repeated-measures analysis of variance (rmANOVA) with dose, time from injection, treatment condition (for noncontingent drug administration), and session number as within-subjects factors and drug identity as a between-subjects factor as appropriate. Any significant rmANOVA main effects were followed with post hoc analysis using Tukey (within-subjects effects) or Sidak (between groups effects) correction for all possible comparisons. In the self-administration studies, the primary dependent variables were the number of infusions obtained and the drug-associated lever discrimination ratio (drug-associated lever presses/all lever presses). Priming infusions were very uncommon (for MDPV rats priming included: 1 individual on 10 acquisition sessions, 2 individuals on 2 sessions and 3 individuals on 1 session and 4 individuals on 1 session; of these, a single individual was primed on 12 sessions; for alpha-PVP rats priming included: 1 individual on 5 sessions, 2 individuals on 3 sessions; of these a single individual was primed on 5 sessions); thus, these data were not analyzed. The total number of drug-associated lever responses were analyzed in the progressive-ratio procedure since breakpoints and infusions can violate the assumptions of ANOVA. A Kaplan–Meier survival analysis was used to compare acquisition of alpha-PVP and MDPV under the 0.05 mg/kg/infusion dose (nonacquiring rats right censored at 20 sessions; group differences determined by Mantel–Cox logrank test). The post hoc

tests of the temperature and activity data included all possible comparisons, but the reporting of results focuses on logical comparisons of interpretive interest as in prior studies of the effects of THC, MDMA, methamphetamine, MDPV, and mephedrone (Aarde et al. 2013b; Miller et al. 2013a, b; Taffe et al. 2015; Wright et al. 2012) in rats as well as studies of MDMA, methamphetamine, and THC in monkeys (Crean et al. 2006, 2007; Taffe 2011, 2012; Taffe et al. 2006; Von Huben et al. 2007). The first set of comparisons evaluated change from the preinjection baseline within treatment conditions, and the second set compared drug effects with the vehicle condition at corresponding time-points postinjection.

Analyses were conducted using Prism 6 for Windows (v. 6.02; GraphPad Software, Inc, San Diego CA, USA) or StatView (SAS Institute, Inc, Cary, NC, USA). Graphs were generated with Excel (Microsoft, Redmond, WA, USA) and figures created in Canvas (v.12; ACD Systems of America, Inc, Seattle, WA, USA).

Results

Self-administration

Experiment 1: establishing a training dose of alpha-PVP

Acquisition A group of rats ($N=9$) was trained to self-administer alpha-PVP with 0.1 mg/kg delivered for each infusion; seven rats' catheters remained patent through the 20 session interval. As shown in Fig. 2, mean infusions obtained were essentially stable for 20 sessions; however, the drug lever discrimination improved substantially across sessions. The one-way ANOVA did not confirm any significant effect of training session for infusions obtained, but there was a main effect of training session on lever discrimination [$F(6,19)=6.007$; $P<0.0001$]. Post hoc comparisons confirmed that discrimination was higher in sessions 7 and 9–20 than 1 and 3 as well as higher in sessions 14–19 than 20. One rat responded for an unusually elevated number of infusions on four sessions (see the large deviations in mean and variance on sessions 7, 11, 12, and 17), which may represent isolated voluntary binge behavior; however, it cannot be excluded that this may have been essentially involuntary repetitive responding (stereotypy).

Fixed-ratio dose–response Five rats remained patent through the FR series. Analysis of the dose substitution study (Fig. 2, lower panel) confirmed that there was a significant effect of dose on mean infusions obtained [$F(1.854, 7.415)=141.2$; $P<0.0001$] as well as lever discrimination [$F(1.383, 5.532)=16.50$; $P<0.01$]. The post hoc test confirmed that infusions were significantly different between all dose pairs except vehicle vs 0.05 and 0.1 vs 0.25. A post hoc test also

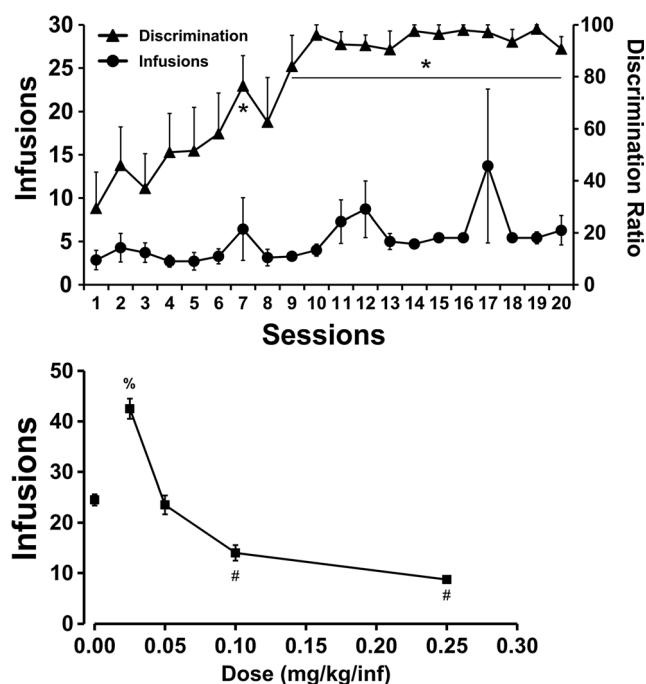


Fig. 2 Acquisition of alpha-PVP (0.1 mg/kg/infusion) self-administration. Mean ($\pm$ SEM) infusions and drug-lever discrimination ratios during acquisition (upper panel; $N=9$) and infusions obtained during dose substitution (lower panel; vehicle, 0.025, 0.05, 0.1, 0.25 mg/kg/infusion; $N=5$) under a fixed-ratio 1 response requirement. Significant differences in lever discrimination from the first and third acquisition session are indicated by asterisk in the upper panel. In the lower panel, significant differences from vehicle, 0.025, and 0.05 are indicated by number sign, and a significant difference from vehicle and 0.05 mg/kg/infusion is indicated by percent sign

confirmed that discrimination ratio was significantly lower in the vehicle condition (mean, 66.1 %; SEM, 7.5) compared with each active dose (means, 95.6–98.8), except 0.05 mg/kg/infusion (mean, 97.3; SEM, 1.9).

Experiment 2: comparison of alpha-PVP with MDPV

Comparison of our prior results for MDPV IVSA (Aarde et al. 2013b) with the alpha-PVP acquisition (fewer asymptotic infusions) and fixed-ratio dose-substitution data (nearly identical curves) in the first experiment recommended a 0.05 mg/kg/infusion training dose of alpha-PVP for direct comparison with MDPV at the same training dose. Likewise, the finding that all rats in this preliminary study reached three or more infusions of alpha-PVP (0.1 mg/kg/infusion) matches well with a recent MDPV IVSA study (Aarde et al. 2015) in which acquisition criteria of sustained intake six or more infusions at 0.05 mg/kg/infusion were met.

Acquisition of intravenous self-administration

The mean number of infusions of MDPV and alpha-PVP obtained, as well as the discrimination ratios (drug-paired lever

responses/all lever responses), is depicted in Fig. 3. Subject loss from the original groups included two due to loss of catheter patency (one from each group) and eight due to illness (six MDPV, two alpha-PVP), thus affecting completed group sizes for MDPV ($N=11$) and alpha-PVP ($N=15$). The MDPV group was further reduced by two due to a failure to make a priori acquisition criteria (see below). Infusions of alpha-PVP and MDPV systematically increased over the 20-session acquisition period as reflected in a significant main effect of session [$F(19, 418)=9.710$; $P<0.0001$] and interaction [$F(19, 418)=3.250$; $P<0.0001$], but no effect of drug identity, in the ANOVA. Post hoc analysis confirmed that significantly more infusions of MDPV were taken in session 12 compared with sessions 2; in sessions 9, 11–12, and 19 compared with session 3; in sessions 12 and 19 compared with session 4; and in session 19 compared with session 5. For alpha-PVP rats, significantly more infusions were obtained in sessions 11–20 relative to session 4; in

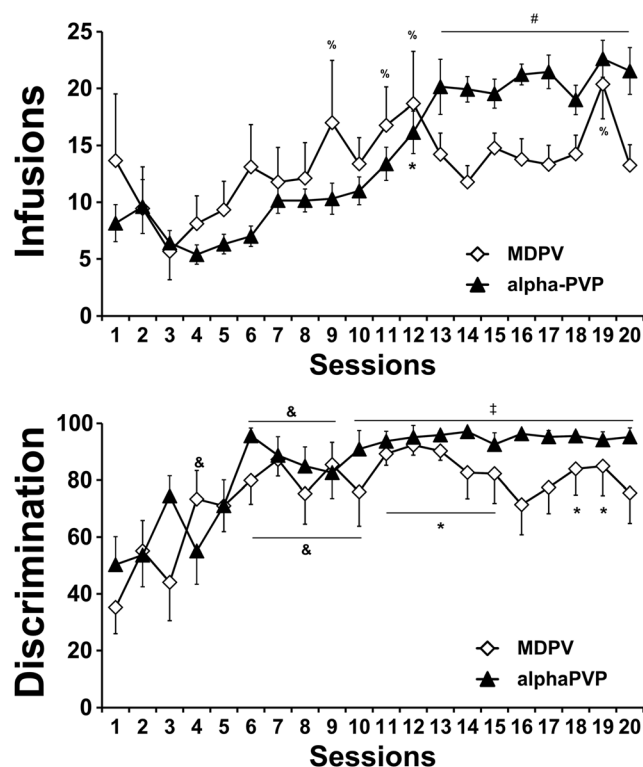


Fig. 3 Comparing the acquisition of alpha-PVP (0.05 mg/kg/infusion) and MDPV (0.05 mg/kg/infusion) self-administration. Mean ($\pm$ SEM) infusions obtained per session (upper panel) and drug-paired lever discrimination ratios (lower panel) across sessions of acquisition of alpha-PVP ($N=15$) and MDPV ($N=9$) self-administration for those individuals who met acquisition criteria. Significant differences from the first six sessions is indicated by number sign; from the first, second, and fourth sessions by double dagger; versus the first and third sessions by asterisk; compared with the first session by ampersand and from the third session by percent sign. See “Results” for additional significant differences

sessions 12–20 relative to sessions 1, 3, 5, and 6; and in sessions 13–20 relative to sessions 2 and 8. Intermittent differences from sessions 7, 9, and 10 were also confirmed within the block of sessions from 13 to 20 for the alpha-PVP group. No significant differences between groups were confirmed for any session.

Drug-lever discrimination also significantly increased across the 20-day acquisition interval, as reflected in a significant main effect of session [$F(19, 418)=9.642$; $P<0.0001$], but no effect of drug identity or of the interaction in the ANOVA. Post hoc analysis confirmed that MDPV lever discrimination was significantly higher in sessions 4, 6–15, and 17–20 compared with the first session and in sessions 7, 9, 11–15, and 18–19 compared with the third session. Lever discrimination in the alpha-PVP group was also significantly improved compared with the first (sessions 6–20), second (sessions 6–20), fourth (sessions 6–7, 10–20), and fifth (session 18) sessions.

Two of the 11 MDPV rats that completed the acquisition interval failed to make a priori acquisition criteria of intake six or more infusions (Aarde et al. 2015) for three sessions. To determine if there was any difference in the group rate of acquisition, rats were defined as having acquired self-administration on the first of three sequential days in which they responded for six or more infusions; these data were subjected to a survival analysis with rats that failed to make criteria censored at 20 sessions (Fig. 4). There was no significant difference between groups even when using the most liberal logrank (Mantel–Cox) procedure [$\chi^2(1, N=24)=0.721$; $P=0.396$]. Exploration with an even more stringent criterion of sustaining six or more infusions without any subsequent sessions below six infusions only reduced the terminal acquisition percentages to 87 % of the alpha-PVP group and 73 % of the MDPV group, but again, there was no significant difference in group acquisition.

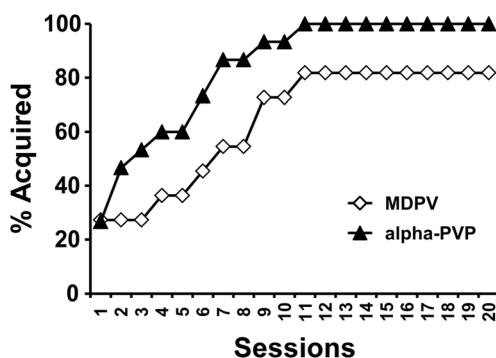


Fig. 4 Percent of subjects acquiring self-administration. The cumulative percentage of rats that reached acquisition criteria are depicted as a function of session and the drug available (MDPV, $N=11$; alpha-PVP, $N=15$) over the 20-session acquisition period. Rats were defined as having acquired self-administration on the first of three sequential sessions for which six or more infusions were acquired

Progressive-ratio dose–response

Two sequential rounds of the PR dose substitution were completed in MDPV-trained ($N=9$) and alpha-PVP-trained ($N=12$) rats (Fig. 5). The analysis of drug-associated lever pressing confirmed a significant main effect of dose [$F(6, 114)=7.20$; $P<0.0001$] but not of drug identity nor any interaction between factors. For reference, the mean infusions obtained ranged from 3.7 (0.018 mg/kg/infusion MDPV) to 7.5 (0.18 mg/kg/infusion alpha-PVP) in this study.

Post hoc comparisons confirmed that the alpha-PVP rats emitted significantly less lever pressing when the per-infusion dose was 0.018 compared with 0.1, 0.18, and 0.32 mg/kg/infusion doses. They also pressed significantly less for 0.032 mg/kg/infusion compared with the 0.018 mg/kg/infusion sessions. The MDPV rats responded significantly less on the drug-associated lever when the dose was 0.018 mg/kg/infusion compared with the 0.1 and 0.18 mg/kg/infusion conditions; in addition, lever pressing differed between 0.18 and 0.56 mg/kg/infusion condition. All rats for both drugs reached a breakpoint before the end of their session for doses at or below 0.1 mg/kg, thus confirming that the selection of the PR schedule worked as intended.

Experiment 3: physiological effects

Locomotor activity Locomotor activity was altered by intraperitoneal administration of both alpha-PVP and MDPV as depicted in Fig. 6. The initial analysis including all treatment conditions (vehicle, 1.0, 5.6, and 10.0 mg/kg of each of alpha-PVP and MDPV) confirmed a significant main effect of drug treatment condition [$F(6, 42)=3.154$; $P=0.0121$], of time postinjection [$F(12, 84)=10.67$; $P<0.0001$] and of the interaction between factors [$F(72, 504)=1.664$; $P=0.0010$]. Post hoc analysis confirmed that the 1.0 mg/kg alpha-PVP activity was significantly different after vehicle (30 min postinjection)

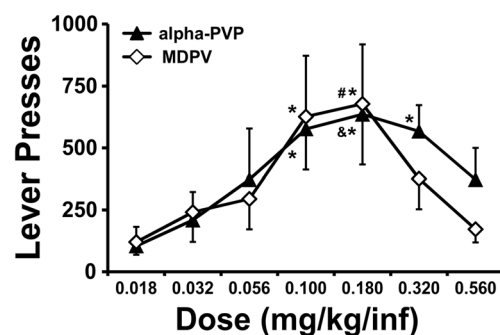


Fig. 5 Progressive ratio dose substitution. Mean ($\pm$ SEM) drug-associated lever presses during the self-administration of alpha-PVP ($N=12$) or MDPV ($N=8$) under a progressive-ratio schedule of reinforcement. Significant differences from the 0.018 mg/kg/infusion dose condition are signified by asterisk, from the 0.032 dose by ampersand and differences from the 0.56 dose by number sign

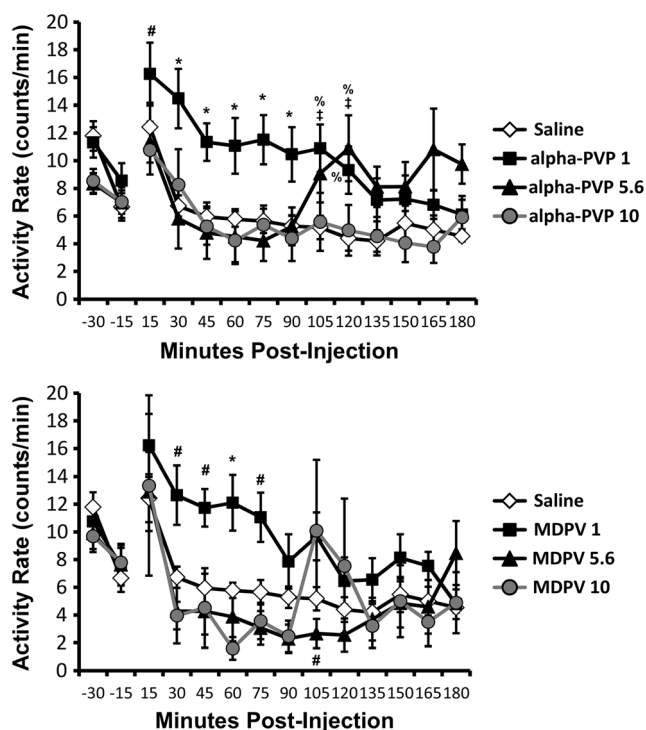


Fig. 6 Locomotor activity. Mean ($N=8$; $\pm$ SEM) activity rate after alpha-PVP (upper panel) or MDPV (lower panel) was administered i.p. The vehicle condition is the same in both panels as all drug doses were randomized with a single vehicle condition. Significant differences from vehicle alone are indicated by *percent sign*; from vehicle and the other two active doses by *asterisk*; from the other two active doses by *number sign*; and from the 10 mg/kg dose is indicated by *double dagger*

compared with the 5.6 mg/kg (30 and 75 min postinjection) and 10.0 mg/kg (60 and 165 min postinjection) alpha-PVP conditions. Activity after 1.0 mg/kg alpha-PVP differed significantly from the higher MDPV doses at several time points but did not differ from activity following the 1.0 mg/kg MDPV dose at any time postinjection. The post hoc test also confirmed that activity following the 1.0 mg/kg MDPV dose differed significantly from activity after the 5.6 and 10.0 mg/kg doses of MDPV 30–75 min postinjection. Activity after 5.6 mg/kg MDPV differed from both the 1.0 and 10.0 mg/kg conditions 105 min after administration.

Body temperature Body temperature was altered by both MDPV and alpha-PVP as depicted in Fig. 7. The analysis including all treatment conditions (vehicle, 1.0, 5.6, and 10.0 mg/kg of each of alpha-PVP and MDPV) confirmed a significant main effect of drug treatment condition [$F(6, 42)=4.463$; $P=0.0014$], of time postinjection [$F(12, 84)=13.16$; $P<0.0001$], and of the interaction between factors [$F(72, 504)=1.756$; $P<0.0005$]. The post hoc test confirmed that body temperature was lower compared with the vehicle condition and the pretreatment baseline (–15 min) within condition after 5.6 mg/kg (45–60 min postinjection) and 10 mg/kg (45–180 min) alpha-PVP as well as after 5.6 mg/kg

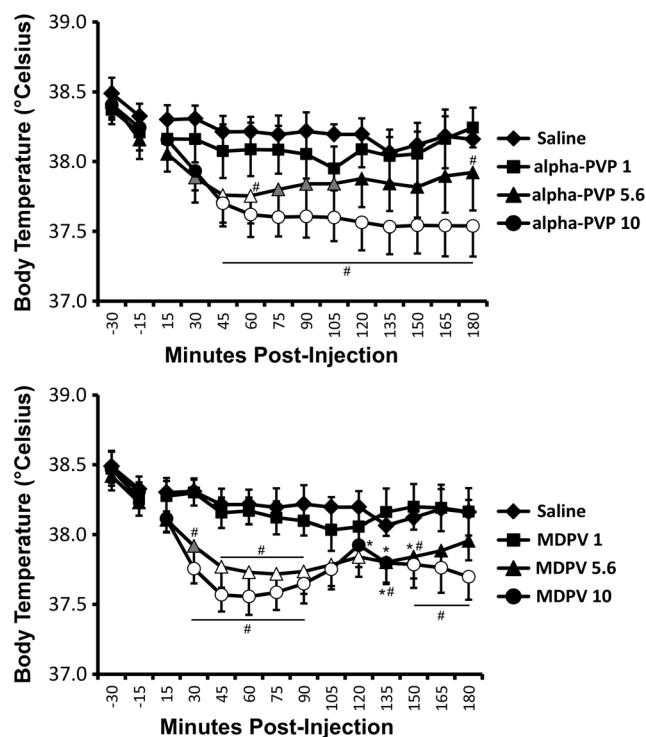


Fig. 7 Thermoregulation. Mean ($N=8$; $\pm$ SEM) body temperature after alpha-PVP (upper panel) or MDPV (lower panel) was administered i.p. The vehicle condition is the same in both panels as all drug doses were randomized with a single vehicle condition. *Shaded figures* indicate a significant difference from vehicle at the respective timepoint. *Open figures* indicate a significant difference from vehicle at the respective timepoint and the baseline within treatment condition (15 min prior to injection). Significant differences from baseline (only) are indicated with *asterisk* and differences from the 1.0 mg/kg dose (within compound) with *number sign*

(45–120 min) and 10 mg/kg (30–105 and 150–180 min) MDPV. In addition, body temperature was significantly reduced relative to the vehicle condition (but not the baseline after 5.6 mg/kg alpha-PVP (30 and 75–105 min postinjection) and 5.6 mg/kg MDPV (30 min postinjection). Within a drug compound, temperature was significantly lower than the 1.0 mg/kg dose after 5.6 mg/kg (60 and 180 min postinjection) and 10.0 mg/kg (45–180 min) alpha-PVP as well as after 5.6 mg/kg (30–90 and 135–150 min) and 10.0 mg/kg (30–90 and 150–180 min) MDPV. The only time point in which temperature differed between equal doses of MDPV and alpha-PVP was 120 min after the administration of 10 mg/kg. Finally, body temperature was significantly different from the pretreatment baseline (–15 min) after 5.6 mg/kg (135–150 min postinjection) or 10.0 mg/kg (120–135 min) MDPV.

Discussion

The results of this investigation show that alpha-PVP is similar to MDPV in both potency and efficacy as a reinforcer as

assessed with intravenous self-administration in rats. The rates of group acquisition under equal training doses (0.05 mg/kg/infusion) did not significantly differ in terms of a Kaplan–Meier analysis of sessions to criteria, although intake was higher and more stable for alpha-PVP than MDPV at the end of the acquisition period as confirmed by a significant interaction of sessions and training drug. Lever discrimination was also more consistently higher in the alpha-PVP trained group, which might indicate a more consistently reinforcing effect. Together, these outcomes may possibly indicate increased addiction liability for alpha-PVP over MDPV on population-wide basis. It should be noted that two groups trained on MDPV (0.05 mg/kg/infusion) in another recent study (Aarde et al. 2015) from this laboratory generated mean intakes in the final sessions that were comparable to the present MDPV group. The similarity of the drugs was particularly apparent in the nearly identical dose–response functions generated in a progressive-ratio procedure following initial acquisition. Indirect comparison of the 0.1 mg/kg/infusion trained alpha-PVP group's responding under FR1 dose substitution with prior results for MDPV (Aarde et al. 2013b) also show this similarity, although a direct comparison would be required for a definitive conclusion. Likewise, a similar potency and efficacy of the two drugs was observed on locomotor stimulation and thermoregulatory disruption (Figs. 6 and 7). Thus, the human abuse liability of these two compounds is predicted to be nearly indistinguishable, despite the presence of the 3,4-methylenedioxy motif, which differentiates MDPV. This moiety is associated with significantly reduced potency and efficacy as a reinforcer when added to methamphetamine to produce MDMA as has been shown in prior studies (De La Garza et al. 2007; Feduccia et al. 2010; Kitamura et al. 2006; Schenk et al. 2007). The present findings show, therefore, that inferences about the role of this structural motif based solely on comparing reinforcer potency or efficacy of methamphetamine with MDMA may be misleading, particularly after regular use has been established. It also raises the possibility that relative DA/5HT effects of compounds that act (only) as transporter inhibitors are less important compared with the compounds that release monoamines. This shows that it is imperative to evaluate the abuse liability of novel cathinone derivatives directly with in vivo models since reliance on structure–activity relationships established with the amphetamines are poorly predictive. The amphetamines act as monoamine transporter substrates and monoamine releasers, whereas MDPV and alpha-PVP appear to only function as transporter blockers (Baumann et al. 2013; Simmler et al. 2013), likely due in part to the presence of the pyrrolidine ring (Kolanos et al. 2013). It remains to be further determined if the unimportance of the methylenedioxy motif is associated with cathinones or with the subset of cathinones that act only as transporter inhibitors but not substrates or monoamine releasers.

The reported potency differences between MDPV and alpha-PVP on dopamine transporter inhibition and mouse locomotor assays in a prior study (Marusich et al. 2014) predicted that MDPV would be slightly more potent as a reinforcer, and yet no such effect was found. Indeed, the most striking feature of the PR study was the similarity of the curves. This observation may reflect the (low) affinity for the SERT found for MDPV contrasted with essentially no SERT affinity for alpha-PVP (Baumann et al. 2013; Marusich et al. 2014) given that DAT/SERT inhibition ratio is related to potency in intravenous self-administration (Roberts et al. 1999; Wee et al. 2005). Similarly, it is that case that rat self-administration of MDMA is marked by intersubject inconsistency (Dalley et al. 2007) with up to half of a sample failing to meet acquisition criteria (Colussi-Mas et al. 2010; Oakly et al. 2014; Schenk et al. 2007); this is one reason that discrimination ratio is used in this study as a dependent variable rather than a screening criterion. Therefore, it may be that the modest SERT affinity of MDPV counteracts the increased DAT potency relative to alpha-PVP, thereby rendering the two compounds very similar in IVSA potency in vivo. Alternately, it may be that there is no functional effect on SERT at self-administered doses and that, therefore, the small differences in DAT potency as determined in vitro for MDPV do not translate to discernible potency differences for intravenous self-administration.

The locomotor stimulant effects of alpha-PVP were also very similar to those of MDPV, particularly in the finding that the peak locomotor stimulant dose was 1.0 mg/kg and effects lasted about 2 h after injection and the lack of statistical differences between drugs at identical doses. The MDPV results were themselves roughly consistent with those previously reported for rats (Aarde et al. 2013b; Huang et al. 2012) in terms of duration of action and dose relationships. In contrast, peak locomotor-stimulant doses in mouse have been reported at 10–30 mg/kg, i.p., with effects lasting up to 6 h postadministration (Fantegrossi et al. 2013; Gatch et al. 2013; Marusich et al. 2012). It was also notable that the 5.6 mg/kg dose of alpha-PVP engendered increased activity in a sustained manner after about 105 min postinjection. This multiphasic pattern is similar to the effects of higher doses of methamphetamine in inducing initial stereotyped movement (and low locomotion), which is replaced after a few hours with increased locomotion as drug levels subside (Segal and Kuczenski 1997). This pattern is similar to one previously reported for MDPV using a wheel-running assay; however, in that case, the suppression/rebound was observed after 1.0 mg/kg MDPV, but not 5.6 mg/kg, within a 1-h observation interval (Huang et al. 2012). Qualitatively, there was some indication that the higher two doses of MDPV may have suppressed activity below that observed after vehicle injection. Combining this finding with the rebound of activity 2 h after injection of 5.6 mg/kg alpha-PVP may indicate

slightly increased potency of MDPV in terms of inducing stereotyped behavior (which was not directly measured in this study).

As noted above, MDPV has been shown to elevate body temperature in some cases of human medical emergency and/or death (Borek and Holstege 2012; Froberg et al. 2015; Kesha et al. 2013). In this study, a modest, but consistent, alteration in body temperature ($\sim 0.75^\circ\text{C}$) was observed after either MDPV or alpha-PVP; this is similar to the magnitude of change produced by methamphetamine, 4-methylmethcathinone (mephedrone), or MDPV in our prior studies (Aarde et al. 2013b; Miller et al. 2013a, b; Wright et al. 2012) and a finding for MDPV in mice (Fantegrossi et al. 2013). The effects were dose dependent and lasted up to 3 h after dosing. The temperature change in the *hypothermic* direction was what would be predicted for rats evaluated under the 21°C ambient temperature condition of this study based on prior findings for MDMA (Malberg and Seiden 1998), methamphetamine (Gilpin et al. 2011; Miller et al. 2013b; Myles et al. 2008), and mephedrone (Aarde et al. 2013a; Miller et al. 2013a; Wright et al. 2012) as well as the nonsubstrate, transporter inhibitor cocaine (Cox and Lee 1979), and dopamine direct agonist apomorphine (Brown et al. 2007). Differences with prior reports of hyperthermic effects of MDPV in rats are likely due to ambient temperature, strain, and especially light cycle differences (King et al. 2014; Kiyatkin et al. 2015).

There has been a great deal of confusion in the popular media and lay public resulting from grouping a diverse array of new and emerging substituted cathinone drugs under the single label “bath salts.” 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, 2013; Simmler et al. 2013, 2014) and even locomotor/subjective properties of these diverse stimulants. So far, only limited self-administration data are available for mephedrone (Aarde et al. 2013a; Hadlock et al. 2011; Motbey et al. 2013), MDPV (Aarde et al. 2013b; Watterson et al. 2014b), methylone (Watterson et al. 2012), and now alpha-PVP. These data have shown that inferences about reinforcer efficacy and abuse liability based on molecular structure alone are not sufficient. Some entities such as MDPV and alpha-PVP are restricted monoamine transporter inhibitors, not monoamine releasers like many amphetamine and cathinones (Marusich et al. 2014; Simmler et al. 2013, 2014) and have been shown to be highly potent and efficacious reinforcers (Aarde et al. 2013b; Watterson et al. 2014b). Other entities such as mephedrone have been demonstrated to have unexpectedly high abuse liability in rodent self-administration assays (Aarde et al. 2013a; Hadlock et al. 2011), compared with what would be expected based on the MDMA-like neurochemical response in the nucleus accumbens (Baumann et al. 2012; Kehr et al. 2011; Wright et al.

2012); limited data for methylone are mixed (Creehan et al. 2015; Watterson et al. 2012). As we show here in the direct comparison of alpha-PVP with MDPV, the 3,4-methylenedioxy motif does not strongly predict reduced reinforcer efficacy. In conclusion, this study predicts that the human abuse liability of alpha-PVP will be significant, and similar to that of MDPV.

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