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Conditioned Taste Avoidance, Conditioned Place Preference and Hyperthermia induced by the Second Generation 'Bath Salt' α -pyrrolidinopentiophenone (α -PVP)

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Abstract

Background— α -pyrrolidinopentiophenone (α -PVP) has been reported to be rewarding in a variety of pre-clinical models. Given that a number of drugs of abuse have both rewarding and aversive effects, the balance of which influences addiction potential, the present study examined the aversive properties of α -PVP by assessing its ability to induce taste avoidance. This assessment was made in a combined taste avoidance/place conditioning design that also allowed an evaluation of the relationship between α -PVP's aversive and rewarding effects.

Methods—Male Sprague-Dawley rats were exposed to a novel saccharin solution, injected with one of four doses of α -PVP (0, 0.3, 1.0 and 3.0 mg/kg) (IP) and placed on one side of a place conditioning apparatus. The next day, they were injected with vehicle, given access to water and placed on the other side. Following four conditioning cycles, saccharin avoidance and place preferences were then assessed. The effects of α -PVP on body temperature were also examined.

Results— α -PVP induced dose-dependent taste avoidance as well as significant increases in time spent on the drug-paired side (although this effect was not dependent on dose). α -PVP also induced dose- and time-dependent hyperthermia.

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Contributors

KHN and ALR participated in the design and coordination of the study, drafted the manuscript and performed the statistical analyses. KHN, BJH and MMC performed all the data collection. KCR (Drug Design and Synthesis Section) synthesized the α -PVP. All authors contributed to and approved the final manuscript.

Conflict of interest

No conflict declared.

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Conclusions— α -PVP induced significant taste avoidance whose strength relative to the psychostimulants methylenedioxypyrovalerone (MDPV) and cocaine paralleled their relative binding to the dopamine transporter. Similar to other drugs of abuse, α -PVP has both aversive and rewarding effects. It will be important to assess how various experiential and subject variables impact these effects and their balance to predict abuse liability.

Keywords

α -PVP; conditioned taste avoidance; conditioned place preference; hyperthermia; bath salts

1. Introduction

Drugs of abuse have both rewarding and aversive effects (Cunningham, 1979; Turenne et al., 1996; Verendeev and Riley, 2011), and it is the balance of these two affective properties that impact their use and abuse (Colechio et al., 2014; Riley et al., 2009; Stolerman, 1985; Wise et al., 1976). One class of drugs only beginning to be examined in this context are the synthetic cathinones (Al-Juhaishi et al., 2012; Busardo et al., 2015; Kalix, 1992; Patel, 2000). Among these newly emerged compounds is α -pyrrolidinopentiophenone (α -PVP; aka “gravel”; “flakka” or “five dollar insanity”), one of the most popular “second-generation bath salts” (Marinetti and Antonides, 2013; Marusich et al., 2014). α -PVP, despite being known as a ‘second-generation’ bath salt, is actually the structural parent of the ‘first generation’ bath salt MDPV and has many characteristics in common (Aarde et al., 2015; Glennon and Young, 2016; Katselou et al., 2015; Marusich et al., 2014). Although α -PVP has not received the same attention as MDPV (for reviews on the behavioral and biochemical actions of MDPV, see Bauman et al., 2013; Glennon and Young, 2016; King and Riley, 2016; Meltzer et al., 2006; Simmler et al., 2013), some characterization has recently begun. For example, Aarde and colleagues (2015) reported that in rats α -PVP was similar to MDPV in both potency and efficacy in supporting intravenous self-administration (see also Schindler et al., 2015). Additionally, Watterson et al. (2014) demonstrated that in rats α -PVP dose-dependently decreased ICSS thresholds in a discrete trials current threshold procedure (Marusich et al., 2016). Consistent with this, Gatch et al. (2015) reported that in mice α -PVP induced significant dose-dependent conditioned place preferences.

As noted above, most drugs of abuse have both rewarding and aversive effects and their balance impacts abuse potential. Although α -PVP’s rewarding effects have been examined, little is known of its aversive effects. Previous work has reported that a variety of CNS stimulants (including MDPV; see King et al., 2014; King et al., 2015; Merluzzi et al., 2013) are effective in inducing dose-dependent conditioned taste avoidance (CTA), a behavioral index of the aversive effects of drugs (Garcia and Ervin, 1968; Lin et al., 2016; Riley and Tuck, 1985). The taste avoidance procedure is one in which animals are given access to a novel taste and then injected with a drug. As a consequence of this pairing, consumption of the drug-paired taste is suppressed, a suppression generally described to be function of the drug’s aversive effects (Garcia and Ervin, 1968; Revusky and Garcia, 1970; Riley and Tuck, 1985; Rozin and Kalat, 1971; for a review on the history of CTA, see Freeman and Riley, 2009). The present study began the initial characterization of the aversive effects of α -PVP by examining its ability to induce taste avoidance.

Although many drugs have been reported to induce taste avoidance, the mechanism underlying such effects in general remains unknown (Gamzu et al., 1985; Hunt and Amit, 1987; for reviews, see Lin et al., 2016; Verendeev and Riley, 2012). The limited work with cocaine suggests that dopamine activity may mediate, in part, its aversive effects. For example, a variety of dopamine antagonists block cocaine-induced taste avoidance (Freeman et al., 2005b; Hunt et al., 1985; Serafine et al., 2011, 2012) and cocaine's aversive effects are significantly attenuated by a prior history with other dopamine-reuptake inhibitors, an effect generally interpreted to be a function of cross-tolerance between the compounds (Berman and Cannon, 1974; Freeman et al., 2005b; LeBlanc and Cappell, 1974; Serafine and Riley, 2010; for a review, see Riley and Simpson, 2001). It is interesting in this context that MDPV appears to be roughly 10 times more potent than cocaine in inducing taste avoidance (Freeman et al., 2005a b; King et al., 2014, 2015; Marusich et al., 2014; Merluzzi et al., 2013; Serafine et al., 2012; Woloshchuk et al., 2016). This relative potencies of MDPV and cocaine in inducing taste avoidance parallel their relative efficacy at blocking DA reuptake (MDPV: IC₅₀ values of 4.1 ± 0.5 nM; cocaine: IC₅₀ values of 211 ± 19 nM). Although α -PVP also inhibits the reuptake of dopamine, its relative efficacy is much more similar to MDPV than cocaine (α -PVP: IC₅₀ values of 12.8 ± 1.2 nM; Marusich et al., 2014). If dopamine activity mediates the aversive effects of α -PVP in a manner similar to that for cocaine (and MDPV; see Woloshchuk et al., 2016), it might be expected to induce taste avoidance to a degree comparable to MDPV and at doses less than those needed to induce avoidance with cocaine and doing so with fewer conditioning trials.

As noted above, Gatch and colleagues (2015) have previously reported CPP with α -PVP in mice; however, species differences in place preference conditioning have been noted with other drugs (see Chaperon et al., 1998; Cunningham et al., 1993; Hempel et al., 2016; Hutcheson et al., 1998; Saludo-Pena et al., 1997; Valjent and Maldonado, 2000). Accordingly, place preference conditioning was also assessed in the present experiment. Specifically, rats were allowed access to a novel saccharin solution and injected with one of four doses of α -PVP (0, 0.3, 1.0 and 3.0 mg/kg) prior to being placed in a distinct environment, a procedure used by our laboratory (Hempel et al., 2016; King et al., 2015; Pomfrey et al., 2015; Simpson and Riley, 2005; Verendeev and Riley, 2011) and others (Brockwell et al., 1991; Sherman et al., 1980; Wang et al., 2010) to simultaneously assess the aversive (CTA) and rewarding (CPP) effects of a host of drugs of abuse.

Core body temperature was also examined in response to α -PVP. Increases in body temperature is commonly observed with a number of psychostimulants (for MDMA, see Dafters and Lynch, 1998; for methamphetamine, see Fukumura et al., 1998; for cocaine, see Cappon et al., 1998; for MDPV, see Fantegrossi et al., 2013; King et al., 2014; Kiyatkin et al., 2015; Merluzzi et al., 2013). Interestingly, Aarde and colleagues (2015) have recently reported *hypothermia* in rats receiving 5.6 and 10.0 mg/kg of either α -PVP or MDPV. To extend these assessments of temperature changes with α -PVP, following the abovementioned behavioral tests, subjects were injected with α -PVP and core body temperature was examined. This assessment also allowed for an initial examination of the relationship of taste avoidance conditioning to changes in temperature (see Cunningham et al., 1992; Merluzzi et al. 2013).

2. General Methods

2.1. Subjects

The subjects were 33 experimentally naïve male Sprague-Dawley rats (Envigo, Indianapolis, IN). Rats entered the animal research facility at American University on postnatal day (PND) 23 and were allowed to mature undisturbed with the exception of weekly weight assessments from PND 23 through PND 83. Beginning on PND 83, animals were weighed daily to index health status and to reduce handling stress during the experimental procedures. Subjects were 90 days old and weighed between 321 and 445 grams at the beginning of the study. All procedures adhered to the Guidelines for the Care and Use of Laboratory Animals (National Research Council, 2011) and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (2003) and were approved by the Institutional Animal Care and Use Committee at American University.

2.2. Drugs and solutions

Racemic α -pyrrolidinopentiophenone HBr (α -PVP) (synthesized at the Drug Design and Synthesis Section, NIDA) was dissolved in isotonic saline (0.9%) and injected intraperitoneally (IP) at 0.3, 1.0 and 3.0 mg/kg. Concentration was held constant across dose groups (1 mg/ml), and animals were injected at a volume of 1 ml/kg. Isotonic saline (vehicle) was administered to controls at the same volume. Each drug (and vehicle) solution was prepared daily and was passed through a 0.2 μ m filter prior to injection. Saccharin (sodium saccharin, Sigma) was prepared as a 1g/l (0.1%) solution in tap water.

2.3. Apparatus

Subjects were housed two per home-cage in OptiRat Plus cages ($38.9 \times 56.9 \times 26.2$ cm; 1181 cm²). The room in which the cages were located was maintained on a 12-h light/dark cycle (0800 – 2000h) at 23 °C. Unless stated otherwise, food and water were available ad libitum. During habituation, training and testing (see below), animals were transferred to individual hanging, stainless-steel wire mesh test cages ($24.3 \times 19 \times 18$ cm) on the front of which graduated Nalgene tubes could be placed for fluid presentation. Eight identical three-chambered conditioned place preference systems (San Diego Instruments Place Preference System, San Diego, CA) were employed for place preference conditioning (for a detailed description, see Hempel et al., 2016). During training and testing, the procedure room was lit with an 85-watt red light mounted in the ceiling.

2.4. Procedure

2.4.1. Habituation—On PND 90, subjects were deprived of water for 24 hours and on the following day were given 20-min access to tap water in the test cages. Following this, the animals were returned to their home cages. This procedure was repeated for 10 days to allow water consumption to stabilize (drinking within 2 seconds with the average volume of water consumed not increasing or decreasing by more than 2 ml for 3 consecutive days). Fluid was presented in graduated 50-ml Nalgene tubes, and intake was indexed by the difference between pre- and post-consumption volumes.

2.4.2 Pre-Test—Following stable water consumption (PND 101), subjects were given 20-min access to water in the test cages, placed into the conditioned place preference (CPP) apparatus for 15 minutes and allowed to investigate all three chambers freely. Subjects spent an average of 216.2 s on the white side and 398.6 s on the black side of the apparatus. Time spent in the two main chambers was significantly different ($p < 0.05$), indicating that the apparatus was biased with rats preferring the black side over the white. Accordingly, a biased training procedure was employed such that subjects were assigned to receive drug injections on the non-preferred side of the apparatus (Le Foll and Goldberg, 2004; Tzschentke, 1998). Following access to the place preference chambers, animals were returned to their home cages. Chambers were wiped down with a soapyalconox and water solution between animals.

2.4.3. Combined Conditioned Taste Avoidance/Conditioned Place Preference

—On Day 1 of this phase (PND 102), rats were placed in their test cages and allowed 20-min access to a novel saccharin solution. Subjects were matched on saccharin consumption, assigned to four groups such that consumption was comparable and injected (IP) with either 0 (saline vehicle), 0.3, 1.0 or 3.0 mg/kg α -PVP ($n = 8$ –9 per group). This dose range was based on work by Watterson and colleagues (2014) in which 0.1, 0.3, 1.0, 3.0 and 5.0 mg/kg α -PVP were assessed in a discrete trials current intensity threshold ICSS procedure that resulted in significant ICSS threshold reductions at 0.3 and 1.0 mg/kg. There were no significant effects at 0.1 mg/kg and at 3.0 mg/kg (see also Aarde et al., 2015; Marusich et al., 2014), and the highest dose (5.0 mg/kg) induced significant elevations in ICSS thresholds. Given that we were attempting to characterize α -PVP's aversive effects, we began with doses within the range that was behaviorally effective as reported by Watterson et al. Following the injections, they were taken to an adjacent room and placed on the non-preferred side of the CPP apparatus (based on the pretest data for each rat) for 30 minutes. Subjects were then returned to their home cages. On the next day (Day 2), rats were given 20-min access to water in the test cages, injected with vehicle and placed on their initially preferred side for 30 minutes. This two-day cycle was repeated for a total of four times. On Day 9, animals were given 20-min access to tap water and placed in the center gray middle area of the CPP apparatus and given 15 minutes to explore the apparatus freely. Time spent in each area was recorded and evaluated for any changes in side preference relative to the amount of time spent in the chambers on the Pre-Test. The next day, animals were placed in the test cages and given 20-min access to both saccharin and tap water in a final two-bottle avoidance test with no subsequent injections. Consumption of both saccharin and water was recorded after 20 minutes had elapsed. Animals were returned to their home cages with ad libitum water access.

2.4.4 Temperature Assessment—Following a 3 week wash-out period (PND 134), temperature probes (Bio Medic Data Systems, Seaford, DE; Model #IPTT-300) were implanted in each animal. Rats were allowed to recover undisturbed for 1 day, and on PND 136–138 they were weighed and the temperature transponders were scanned to check for proper function. On PND 139 and 140, all subjects were scanned for temperature, weighed and injected with saline (IP). On PND 141, they were handled the same as on PND 139 and 140 except that additional temperature scans were taken 30-, 60-, 120- and 180-min post

injection. The procedure on PND 142 was identical except subjects were randomly injected (IP) with either 0 (vehicle), 0.3, 1.0 and 3.0 mg/kg α -PVP. For each temperature recording, the probe was scanned three times and the three measurements averaged.

2.5. Statistical analyses

2.5.1. Conditioned Taste Avoidance—Saccharin consumption on conditioning trials was analyzed using a 4×4 mixed model ANOVA with the between-subjects factor of Dose (0, 0.3, 1.0 and 3.0 mg/kg α -PVP) and the within-subjects factor of Trial (1–4). In the instance of a significant 2-way interaction, simple effects of Dose at each Trial (multivariate analyses) and Trial at each Dose (multivariate analyses) were assessed. Water consumption on recovery days was similarly analyzed using a 4×4 mixed model ANOVA with the between-subjects factor of Dose Group (0, 0.3, 1.0 and 3.0 mg/kg α -PVP) and the within-subjects factor of Recovery Day (1–4). In the instance of a significant 2-way interaction, simple effects of Dose Group at each Recovery Day (multivariate analyses) and Recovery Day at each Dose Group (multivariate analyses) were assessed.

Percent saccharin of total fluid consumption on the final two-bottle test was analyzed using a one-way ANOVA with the between-subject factor of Dose (0, 0.3, 1.0 and 3.0 mg/kg α -PVP) and Bonferroni-adjusted multiple comparisons. Saccharin preference was calculated as a percentage, whereby volume of saccharin consumed was divided by total volume consumed (water + saccharin). Total fluid volume consumed (water + saccharin) on the final two-bottle test was also examined using a one-way ANOVA with the between-subject factor of Dose (0, 0.3, 1.0 and 3.0 mg/kg α -PVP) and Bonferroni-adjusted multiple comparisons.

2.5.2. Conditioned Place Preference—Time spent on the drug-paired side (DPS) was analyzed using a 4×2 mixed model ANOVA with the between-subject factor of Dose (0, 0.3, 1.0 and 3.0 mg/kg α -PVP) and the within-subject factor of Test (Pre-Test and Post-Test), followed by Bonferroni-corrected multiple comparisons as warranted.

2.5.3. Temperature Assessment—Core body temperature (C) was analyzed using a 4×5 mixed model ANOVA with the between-subjects factor of Dose (0, 0.3, 1.0 and 3.0 mg/kg α -PVP) and within-subjects factor of Time (pre-injection, 30, 60, 120 and 180 min post-injection). In the instance of a significant 2-way interaction, simple effects of Dose at each Time (multivariate analyses) and Time at each Dose (multivariate analyses) were assessed. Significance level for all statistical tests was set to $\alpha = 0.05$.

3. Results

3.1. Conditioned Taste Avoidance

α -PVP produced dose-dependent taste avoidance. The 4×4 mixed model ANOVA on saccharin consumption showed significant effects of Dose [$F(3, 29) = 11.914$, $p = 0.000$] and Trial [$F(3, 87) = 3.077$, $p = 0.032$] as well as a significant Dose $\times$ Trial interaction [$F(9, 87) = 12.024$, $p = 0.000$]. In relation to this interaction, multivariate tests used to evaluate effects of Dose at each Trial revealed significant differences on Trials 2–4 [Trial 2: $F(3, 29) = 8.955$; Trial 3: $F(3, 29) = 19.014$; Trial 4 $F(3, 29) = 16.186$]. Bonferroni-corrected multiple

comparisons indicated that by Trial 2 Group 3.0 drank significantly less saccharin than Groups Vehicle, 0.3 and 1.0. These differences were maintained throughout conditioning (see Figure 1). Multivariate analysis of Trial at each Dose revealed significant effects for Dose 1.0 and 3.0 [1.0 mg/kg: $F(3, 27) = 5.402$; 3.0 mg/kg: $F(3, 27) = 26.509$]. Subsequent pairwise comparisons indicated that Groups Vehicle and 0.3 did not significantly decrease or increase the amount of saccharin consumed over trials. Group 1.0 significantly decreased consumption from Trial 2 to Trial 3. Group 3.0 significantly decreased from Trial 1 to Trials 2, 3 and 4. Group 3.0 also significantly decreased consumption from Trial 2 to Trials 3 and 4, but did not differ between Trials 3 and 4.

The 4×4 mixed model ANOVA on water consumption on Recovery Days revealed significant effects of Dose [$F(3, 29) = 5.721$, $p = 0.003$] and Recovery Day [$F(3, 87) = 18.858$, $p = 0.000$] as well as a significant Dose $\times$ Recovery Day interaction [$F(9, 87) = 5.233$, $p = 0.000$]. In relation to this interaction, univariate tests used to evaluate effects of Dose at each Recovery Day indicated significant differences on Recovery Days 3–4 [Recovery Day 3: $F(3, 29) = 8.786$; Recovery Day 4: $F(3, 29) = 8.722$]. Bonferroni-corrected multiple comparisons indicated that by Recovery Day 3 Group 3.0 drank significantly more water than Groups Vehicle, 0.3 and 1.0. These differences were also evident on Recovery Day 4 (see Figure 2). Multivariate analysis of Recovery Day at each Dose revealed significant effects for Groups Vehicle, 1.0 and 3.0 [Vehicle: $F(3, 27) = 4.090$; 1.0 mg/kg: $F(3, 27) = 3.683$; 3.0 mg/kg: $F(3, 27) = 39.632$]. Subsequent pairwise comparisons indicated that Group 0.3 did not significantly decrease or increase the amount of water consumed over recovery days. Groups Vehicle and 1.0 significantly increased consumption from Recovery Day 2 to Recovery Day 3 ($p = 0.019$ for both groups) but did not significantly differ in consumption levels between any other recovery days. Group 3.0 significantly increased water consumption from Recovery Day 1 to Recovery Days 3 and 4 ($p = 0.000$). Group 3.0 also significantly increased consumption from Recovery Day 2 to Recovery Days 3 and 4 ($p = 0.000$), but did not differ between Recovery Day 1 and 2 or between Recovery Days 3 and 4.

On the two-bottle test, the one-way ANOVA on the percent saccharin consumed revealed a main effect of Dose [$F(3, 29) = 4.531$, $p = .010$]. Subjects in Group 3.0 drank significantly less saccharin than those in Groups Vehicle and 0.3. The percent saccharin consumed by Group 1.0 did not significantly differ from any group (see Figure 3). The one-way ANOVA on the total fluid consumed (water + saccharin) during the final two-bottle test revealed no significant differences between groups [$F(3, 29) = 1.31$, $p = .290$] (data not shown).

3.2 Conditioned Place Preference

A 2×4 mixed model ANOVA on percent of time spent on the drug-paired side revealed a main effect of Test [$F(1, 29) = 21.708$, $p = 0.000$] such that percent time increased from Pre-test to Post-test. There was no effect of Dose [$F(3, 29) = 1.701$, $p = 0.189$] nor a significant interaction between Test and Dose [$F(3, 29) = 1.104$, $p = 0.363$]. Due to the lack of a significant interaction, groups were collapsed across dose (all α -PVP treated subjects) and a 2×2 mixed model ANOVA was run with the between group factor of Drug (α -PVP and Vehicle) and the within-group factor of Test (Pre-test and Post-test). Although the overall

initial interaction was not significant, pairwise comparisons showed that drug-exposed animals (collapsed across dose) significantly increased time spent in the drug-paired chamber from Pre-test to Post-test [$F(1,31)=20.282$, $p=.000$] while time spent on the drug-paired side for Group Vehicle did not significantly change [$F(1,31)= 1.645$, $p=.209$] (see Figure 4).

3.3. Temperature Assessment

α -PVP produced dose-dependent increases in temperature. The 4×5 mixed model ANOVA on temperature revealed significant effects of Dose [$F(3, 29)= 4.876$, $p= 0.007$] and Time [$F(4, 116) = 15.119$, $p= 0.000$], as well as a significant Dose $\times$ Time interaction [$F(12, 116) = 5.538$, $p= 0.000$]. In relation to the interaction, multivariate analysis of Dose at each Time period revealed significant effects at Time 30, 60, 120 and 180 [30: $F(3, 29) = 4.20$, $p= 0.014$; 60: $F(3, 29) = 8.028$, $p= 0.000$; 120: $F(3, 29) = 7.133$, $p= 0.001$; 180: $F(3, 29) = 7.947$, $p= 0.001$]. Specifically, pairwise comparisons indicated that at 30 minutes post-injection, Group 3.0 had a significantly higher temperature than Group Vehicle. At 60 and 120 minutes post-injection, Groups 0.3, 1.0 and 3.0 had significantly higher temperature than Group Vehicle. At 180 minutes post-injection, Groups 1.0 and 3.0 had significantly higher temperatures than Group Vehicle. Multivariate analysis of Time at each Dose revealed significant effects for Dose 1.0 and 3.0 [1.0 mg/kg: $F(4, 26) = 7.391$, $p= 0.000$; 3.0 mg/kg: $F(4, 26) = 12.934$, $p= 0.000$]. Subsequent pairwise comparisons indicated that Groups Vehicle and 0.3 did not show any significant changes in temperature across time intervals. Group 1.0 displayed a significant increase in temperature from pre-injection to the 120 and 180 minute intervals. Group 1.0 also showed a significant increase in temperature from 30 to 120 minutes. Group 3.0 displayed a significant increase in temperature from pre-injection to all other intervals (see Figure 5).

4. Discussion

Given that the rewarding effects of α -PVP have recently been described in a variety of preparations (see above), the present study attempted to provide an initial characterization of its aversive effects in rats as indexed by conditioned taste avoidance learning (see Garcia and Ervin, 1968; Riley et al., 2009; Rozin and Kalat, 1971). Place preference conditioning was concurrently assessed given that the prior demonstration of α -PVP's rewarding effects in the CPP design was with mice and species differences have been reported in this specific assay (see Cunningham et al., 1992; Tzschentke, 2007). Finally, core body temperature was assessed to extend the prior work of Aarde et al. (2015) who reported a *hypothermic* effect with this drug (for hyperthermic effects with other bath salts, see Fantegrossi et al., 2013; King et al., 2014; Kiyatkin et al., 2015; Merluzzi et al., 2013; Ross et al., 2012). As described, α -PVP induced both taste avoidance and place preferences, indicative of its aversive and rewarding effects, respectively. Further, it produced significant increases in body temperature.

In relation to taste avoidance learning, while neither 0.3 nor 1.0 mg/kg α -PVP induced avoidance, significant suppression was evident at the highest dose tested, i.e., 3.0 mg/kg. At this dose, animals reduced consumption of the α -PVP-associated saccharin solution by

approximately 40% on the final one-bottle exposure (relative to the initial baseline). These findings are interesting in light of other work with the bath salt MDPV in this design (King et al., 2014, 2015; Merluzzi et al., 2013; Woloshchuk et al., 2016). For example, Merluzzi et al. (2013) reported that while 1.0 mg/kg MDPV induced relatively weak taste avoidance, 1.8 and 3.2 mg/kg reduced consumption of the drug-paired saccharin solution by approximately 50 and 80%, respectively (see also Woloshchuk et al., 2016; see King et al., 2014 for similar effects in inbred LEW and F344 female rats). The fact that greater avoidance was induced by MDPV (at comparable doses and under similar procedures) suggests that the aversive effects of α -PVP are less potent than MDPV at least as assessed in the taste avoidance design.

As noted above, taste avoidance is induced by a host of other drugs (for reviews, see Gamzu et al., 1985; Hunt and Amit, 1987; Verendeev and Riley, 2011, 2012), including a variety of psychostimulants such as cocaine (Ferrari et al., 1991; Pomfrey et al., 2015), MDMA (Cobuzzi et al., 2014), amphetamine (Verendeev and Riley, 2011), caffeine (Vishwanath et al., 2011) and nicotine (Pomfrey et al., 2015; Shoaib et al., 2002). The work with cocaine is most relevant here in that its neurochemical action is similar to that of α -PVP (and MDPV) in terms of blocking the reuptake of the brain amines (see below). Across many behavioral studies examining cocaine-induced taste avoidance (and under training conditions comparable to those described here), suppression of consumption to levels of 40–50% are not evident generally until doses as high as 18 mg/kg are administered (10 mg/kg fails to induce avoidance; Freeman et al., 2005a b; Serafine et al., 2011, 2012). Suppression to levels of 80 and 90% are not apparent until higher doses (32 and 50 mg/kg) are given. Relative to avoidance induced by α -PVP and MDPV, cocaine appears less potent.

The relative strengths of taste avoidance induced by MDPV, α -PVP and cocaine parallel their relative binding to the brain amine transporters (Aarde et al., 2015; Bauman et al., 2013; Marusich et al., 2014; Simmler et al., 2013). Specifically, MDPV and α -PVP have stronger affinities for DAT and NET compared to cocaine (MDPV: IC₅₀ values of 4.1 ± 0.5 nM and 26 ± 8 nM, respectively; α -PVP: IC₅₀ values of 12.8 ± 1.2 nM and 14.2 ± 1.2 nM, respectively; cocaine: IC₅₀ values of 211 ± 19 nM and 292 ± 34 nM, respectively) (Baumann et al., 2013; Cameron et al., 2013; Eshleman et al., 2013; Marusich et al., 2014; Simmler et al., 2013) while having relatively less affinity for SERT (IC₅₀ values of 3349 ± 305 nM, $>10,000$ nM and 313 ± 17 nM for MDPV, α -PVP and cocaine, respectively). Previous work on cocaine-induced avoidance (see Freeman et al., 2005a; Hunt et al., 1985; Serafine et al., 2011, 2012; though see also Gale, 1984) has implicated DA (and to a lesser extent, NE) in its aversive effects. The fact that stronger avoidance is evident with MDPV and α -PVP that have higher binding affinities to the monoamines is consistent with their role in taste avoidance induced by the bath salts.

The fact that α -PVP induces significant taste avoidance is interpreted here as evidence of its aversive effects in that such avoidance may be biologically relevant given that foods are often sources of poisons and toxins and such avoidance (and learning) is highly adaptive (for a discussion, see Rozin and Kalat, 1971; for a recent review, see Lin et al., 2016). There are other interpretations of such avoidance, however. One possible interpretation of the suppression of consumption is that it reflects less an association of the taste with the aversive effects of α -PVP than some unconditioned suppressive effects that the drug may have on

consumption in general. Although possible, the fact that general fluid consumption on recovery days (immediately after each conditioning trial) was not suppressed suggests there was no general suppression of fluid consumption induced by the drug. As noted, with repeated conditioning for the high dose group, water consumption eventually increased. This effect, however, was not evident until saccharin consumption on conditioning trials was significantly suppressed and likely reflects compensatory intake. Further, fluid consumption on the two-bottle test in which both the drug-paired saccharin solution and water were concurrently presented revealed that only saccharin consumption was suppressed. Total fluid consumption on this test was the same for all groups (including controls).

Again, we are arguing that the suppressed saccharin consumption reflects the aversive effects of α -PVP. It is important to note that while such suppression is generally thought to reflect such effects, Grigson (1997) has argued that taste avoidance under some conditions is a function of the rewarding effects of the drug. According to this perspective, the taste paired with the drug (while rewarding) pales in comparison to the drug itself (in this case α -PVP). This anticipatory contrast results in the reduction of intake of the less preferred drug-paired taste. For this position, the drug's rewarding effects induce the avoidance and these two effects should be directly related in specific assessments (see Verendeev and Riley, 2011; 2012; see also Riley et al., 2009). Interestingly, a number of manipulations that affect taste avoidance learning (and the presumed aversive effects of the drug) have no impact on the drug's rewarding properties (and vice versa). Further, manipulations that increase the drug's rewarding effects have been reported to decrease its ability to induce a taste avoidance (and its presumed aversive effects). Such dissociations have been shown with drug pre-exposure, electrolytic lesions, strain and age comparisons and various other parametric variations (for a discussion, see Verendeev and Riley, 2011; 2012; see also Huang and Hsiao, 2008; Wang et al., 2010).

Although the focus of the present study was primarily to assess the aversive effects of α -PVP, its rewarding effects were concurrently evaluated in the place conditioning design. The fact that α -PVP induced place preferences parallels the work by Gatch and his colleagues (2015) who reported place preference conditioning in mice at 0.3–10 mg/kg, a dose range that encompassed that used here. One major difference between the two assessments was the fact that preferences were not dose-dependent with rats but dose-dependent in mice. Given that species differences in place conditioning have been reported (for ethanol, see Cunningham et al., 1993; for cannabinoids, see Flax et al., 2015; Zimmer et al., 2001), it is certainly possible that the presence or absence of a dose-dependent effect could be a function of the specific species assessed. Although it is clear that in both rats and mice α -PVP appears to have rewarding effects, it is important to note that the specific nature of these effects is open to various interpretations. In the present assessment, animals were injected with α -PVP and placed on their non-preferred side (vehicle injections preceded placement on their preferred side). A production of a place preference in a biased procedure may reflect factors other than reward, such as habituation to the aversive effects of the drug (see Bardo and Bevins, 2000) or some negatively reinforcing effects of the drug such as anxiety reduction (for a review, see Tzschentke, 1998; 2007). Although this latter possibility is unlikely with compounds such as α -PVP which are anxiogenic (Baumann et al., 2013;

Ross et al., 2012; Valente et al., 2014; Xu et al., 2016), it remains unknown what specific subjective effects of the drug support place preference conditioning.

In an effort to extend the work of Aarde et al. (2015) with α -PVP-induced changes in temperature, in the current study rats were injected with α -PVP (IP) and body temperature was recorded for 3 hours (for other reports of hyperthermic effects induced by other bath salts, see Fantegrossi et al., 2013; Gannon et al., 2016; King et al., 2014; Kiyatkin et al., 2015; Merluzzi et al., 2013). Relative to the control group, all three groups injected with α -PVP displayed significant increases in temperature. In addition, the effects on temperature were dose- and time- dependent. For example, significant effects on temperature were seen within 30 minutes post injection at only the high dose of α -PVP (Group 3.0), while at 60 and 120 minutes all α -PVP injected groups displayed significant hyperthermia relative to controls. The duration of the effects also varied with dose as the low dose group (Group 0.3) was no longer different from controls by 180 minutes. When examined over the sampling period only Groups 1.0 and 3.0 increased temperature relative to their own baseline (this was evident at all intervals for Group 3.0 and only at the 120 and 180 periods for Group 1.0), while there were no changes in baseline activity for Groups Vehicle or 0.3. Although these results are not consistent with the hypothermia reported by Aarde et al, it is important to note that the doses of α -PVP used in the present assessment were lower than those used by his group, and it is certainly possible that had higher doses been used hypothermic effects would have been seen (Pechlivanova et al., 2010).

Although the temperature assessment was made primarily to extend the limited work on temperature changes with α -PVP, this analysis also allowed for an initial investigation of any possible relationship between changes in temperature and taste avoidance as has been reported in other assessments with other compounds (see Cunningham et al., 1988; 1992; Ionescu and Burešová, 1977). While no direct statistical comparisons could be made due to the randomization of dose groups between assessments, changes in temperature had no obvious relationship with the ability of α -PVP to induce taste avoidance considering that all doses induced hyperthermia but only the highest dose (3.0 mg/kg) induced significant taste avoidance. These findings parallel our earlier work with MDPV, indicating that hyperthermia is unlikely the basis for the acquired avoidance with either α -PVP or MDPV (see King et al., 2014, 2015; Merluzzi et al., 2013).

While it is clear that α -PVP, like MDPV and other drugs of abuse, has both rewarding and aversive effects, the specific factors influencing these effects remain unknown. For each of these affective properties, a host of subject (sex, age, strain, species) and experiential (dose of drug, drug history, route of administration, drug frequency) factors are known to impact their display (Cunningham et al., 2008; Freeman and Riley, 2009; Riley et al., 2009). Given that the balance of these affective properties is thought to mediate use and abuse potential, it will be important to examine how these factors impact the rewarding and aversive effects of α -PVP and how the relative balance of these effects influence α -PVP intake.

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References

- Aarde SM, Creehan KM, Vandewater SA, Dickerson TJ, Taffe MA. *In vivo* potency and efficacy of the novel cathinone α -pyrrolidinopentiophenone and 3,4-methylenedioxypyrovalerone: self-administration and locomotor stimulation in male rats. *Psychopharmacology*. 2015; 232:3045–3055. [PubMed: 25925780]
- Al-Juhaishi T, Al-Kindi S, Gehani A. Khat: A widely used drug of abuse in the Horn of Africa and the Arabian Peninsula: Review of literature. *Qatar Med J*. 2012; 2:1–6.
- Baumann MH, Partilla JS, Lehner KR, Thorndike EB, Hoffman AF, Holy M, Rothman RB, Goldberg SR, Lupica CR, Sitte HH, Brandt SD, Tella SR, Cozzi NV, Schindler CW. Powerful cocaine-like actions of 3,4-methylenedioxypyrovalerone (MDPV), a principal constituent of psychoactive ‘bath salts’ products. *Neuropsychopharmacology*. 2013; 38:552–562. [PubMed: 23072836]
- Berman RF, Cannon DS. The effect of prior ethanol experience on ethanol-induced saccharin aversions. *Physiol Behav*. 1974; 12:1041–1044. [PubMed: 4832445]
- Brockwell NT, Eikelboom R, Beninger RJ. Caffeine-induced place and taste conditioning: production of dose-dependent preference and aversion. *Pharmacol Biochem Behav*. 1991; 38:513–517. [PubMed: 2068188]
- Busardò FP, Kyriakou C, Napoletano S, Marinelli E, Zaami S. Mephedrone related fatalities: a review. *Eur Rev Med Pharmacol Sci*. 2015; 19:3777–3790. [PubMed: 26502870]
- Cameron K, Kolanos R, Verkariya R, Felice LD, Glennon RA. Mephedrone and methylenedioxypyrovalerone (MDPV), major constituents of “bath salts,” produce opposite effects at the human dopamine transporter. *Psychopharmacology*. 2013; 227:493–499. [PubMed: 23371489]
- Cappon GD, Morford LL, Vorhees CV. Enhancement of cocaine-induced hyperthermia fails to elicit neurotoxicity. *Neurotoxicol Teratol*. 1998; 20:531–535. [PubMed: 9761591]
- Chaperon F, Soubrié P, Puech AJ, Thiébot MH. Involvement of central cannabinoid (CB1) receptors in the establishment of place conditioning in rats. *Psychopharmacology*. 1998; 135:324–332. [PubMed: 9539255]
- Cobuzzi JL, Siletti KA, Hurwitz ZE, Wetzell B, Baumann MH, Riley AL. Age differences in ($\pm$)3,4-methylenedioxymethamphetamine (MDMA)-induced conditioned taste aversions and monoaminergic levels. *Dev Psychobiol*. 2014; 56:635–646. [PubMed: 23775255]
- Colechio EM, Imperio CG, Grigson PS. Once is too much: Conditioned aversion develops immediately and predicts future cocaine self-administration behavior in rats. *Behav Neurosci*. 2014; 128:207–216. [PubMed: 24773440]
- Cunningham CL. Flavor and location aversions produced by ethanol. *Behav Neural Biol*. 1979; 27:362–367. [PubMed: 518463]
- Cunningham CL, Gremel CM, Groblewski PA. Genetic influences on conditioned taste aversion. In: Reilly S, Schachtman TR, editors *Conditioned taste aversion: Behavioral and neural processes*. Oxford University Press; New York, NY, US: 2008. 387–421.
- Cunningham CL, Hawks DM, Niehus DR. Role of hypothermia in ethanol-induced conditioned taste aversion. *Psychopharmacology*. 1988; 95:318–322. [PubMed: 3137617]
- Cunningham CL, Niehus JS, Bachtold JF. Ambient temperature effects on taste aversion conditioned by ethanol: contribution of ethanol-induced hypothermia. *Alcohol Clin Exp Res*. 1992; 16:1117–1124. [PubMed: 1471766]
- Cunningham CL, Niehus JS, Noble D. Species difference in sensitivity to ethanol’s hedonic effects. *Alcohol*. 1993; 10:97–102. [PubMed: 8442898]

- Cunningham CL, Tull LE, Rindal KE, Meyer PJ. Distal and proximal pre-exposure to ethanol in the place conditioning task: tolerance to aversive effect, sensitization to activating effect, but no change in rewarding effect. *Psychopharmacology*. 2002; 160:414–424. [PubMed: 11919669]
- Dafters RI, Lynch E. Persistent loss of thermoregulation in the rat induced by 3,4-methylenedioxymethamphetamine (MDMA or “Ecstasy”) but not by fenfluramine. *Psychopharmacology*. 1998; 138:207–212. [PubMed: 9718291]
- Eshleman AJ, Wolfrum KM, Hatfield MG, Johnson RA, Murphy KV, Janowsky A. Substituted methcathinones differ in transporter and receptor interactions. *Biochem Pharmacol*. 2013; 85:1803–1815. [PubMed: 23583454]
- Fantegrossi WE, Gannon BM, Zimmerman SM, Rice KC. *In vivo* effects of abused “bath salt” constituent 3,4-methylenedioxypyrovalerone (MDPV) in mice: drug discrimination, thermoregulation, and locomotor activity. *Neuropsychopharmacol*. 2013; 38:563–573.
- Ferrari CM, O'Connor DA, Riley AL. Cocaine-induced taste aversions: effect of route of administration. *Pharmacol Biochem Behav*. 1991; 38:267–271. [PubMed: 2057497]
- Flax SM, Wakeford AGP, Cheng K, Rice KC, Riley AL. Effect of norbinaltorphimine on 9-tetrahydrocannabinol (THC)-induced taste avoidance in adolescent and adult Sprague-Dawley rats. *Psychopharmacology*. 2015; 232:3193–3201. [PubMed: 26025420]
- Freeman KB, Konaklieva MI, Riley AL. Assessment of the contributions of Na⁺ channel inhibition and general peripheral action in cocaine-induced conditioned taste aversion. *Pharmacol Biochem Behav*. 2005a; 80:281–288. [PubMed: 15680181]
- Freeman KB, Rice KC, Riley AL. Assessment of monoamine transporter inhibition in the mediation of cocaine-induced conditioned taste aversion. *Pharmacol Biochem Behav*. 2005b; 82:583–589. [PubMed: 16337262]
- Freeman KB, Riley AL. The origins of conditioned taste aversion learning: a historical analysis. In: Schachtman TR, Reilly S, editors *Conditioned Taste Aversion: Behavioral and Neural Processes*. Oxford University Press; New York: 2009. 9–33.
- Fukumura M, Cappon GD, Pu C, Broening HW, Vorhees CV. A single dose model of methamphetamine-induced neurotoxicity in rats: effects on neostriatal monoamines and glial fibrillary acidic protein. *Brain Res*. 1998; 806:1–7. [PubMed: 9739098]
- Gale K. Catecholamine-independent behavioral and neurochemical effects of cocaine in rats. *NIDA Res Monogr*. 1984; 54:323–332. [PubMed: 6099878]
- Gamzu E, Vincent G, Boff E. A pharmacological perspective of drugs used in establishing conditioned food aversions. *Ann NY Acad Sci*. 1985; 443:231–249. [PubMed: 2861773]
- Gannon BM, Williamson A, Suzuki M, Rice KC, Fantegrossi WE. Stereoselective effects of abused “bath salt” constituent 3,4-methylenedioxypyrovalerone in mice: drug discrimination, locomotor activity, and thermoregulation. *J Pharmacol Exp Ther*. 2016; 356:615–623. [PubMed: 26769917]
- Garcia J, Ervin FR. Gustatory-visceral and telereceptor-cutaneous conditioning—adaptation in internal and external milieus. *Commun Behav Biol*. 1968; 1:389–415.
- Gatch MB, Dolan SB, Forster MJ. Comparative behavioral pharmacology of three pyrrolidine-containing synthetic cathinone derivatives. *J Pharmacol Exp Ther*. 2015; 354:103–110. [PubMed: 25998047]
- Glennon RA, Young R. Neurobiology of 3,4-methylenedioxypyrovalerone (MDPV) and α -pyrrolidinovalerophenone (α -PVP). *Brain Res Bull*. 2016; 126:111–126. [PubMed: 27142261]
- Grigson PS. Conditioned taste aversions and drugs of abuse: a reinterpretation. *Behav Neurosci*. 1997; 111:129–136. [PubMed: 9109631]
- Hempel BJ, Wakeford AGP, Clasen MM, Friar MA, Riley AL. Delta-9-tetrahydrocannabinol (THC) history fails to affect THC's ability to induce place preferences in rats. *Pharmacol Biochem Behav*. 2016; 144:1–6. [PubMed: 26905371]
- Huang ACW, Hsiao S. Re-examination of amphetamine-induced conditioned suppression of tastant intake in rats: the task-dependent drug effects hypothesis. *Behav Neurosci*. 2008; 6:1207–1216.
- Hunt T, Amit Z. Conditioned taste aversion induced by self-administered drugs: paradox revisited. *Neurosci Biobehav Rev*. 1987; 11:107–130. [PubMed: 3554039]
- Hunt T, Switzman L, Amit Z. Involvement of dopamine in the aversive stimulus properties of cocaine in rats. *Pharmacol Biochem Behav*. 1985; 22:945–948. [PubMed: 2991950]

- Hutcheson DM, Tzavara ET, Smadja C, Valjent E, Roques BP, Hanoune J, Maldonado R. Behavioural and biochemical evidence for signs of abstinence in mice chronically treated with Δ^9 -tetrahydrocannabinol. *Br J Pharmacol*. 1998; 125:1567–1577. [PubMed: 9884086]
- Ionescu E, Burešová O. Effects of hypothermia on the acquisition of conditioned taste aversion in rats. *J Comp Physiol Psychol*. 1977; 91:1297–1307.
- Kalix P. Cathinone, a natural amphetamine. *Pharmacol Toxicol*. 1992; 70:77–86. [PubMed: 1508843]
- Katselou M, Papoutsis I, Nikolaou P, Spiliopoulou C, Athanaselis S. α -PVP (“flakka”): a new synthetic cathinone invades the drug arena. *Forensic Toxicol*. 2015; 34:41–50.
- King HE, Wakeford A, Taylor W, Wetzell B, Rice KC, Riley AL. Sex differences in 3,4-methylenedioxypyrovalerone (MDPV)-induced taste avoidance and place preferences. *Pharmacol Biochem Behav*. 2015; 137:16–22. [PubMed: 26216834]
- King HE, Wetzell B, Rice KC, Riley AL. 3,4-Methylenedioxypyrovalerone (MDPV)-induced conditioned taste avoidance in the F344/N and LEW rat strains. *Pharmacol Biochem Behav*. 2014; 126:163–169. [PubMed: 25284129]
- King HE, Riley AL. The affective properties of synthetic cathinones: the role of reward and aversion in drug abuse. In: Bauman M, editor *Neuropharmacology of New Psychoactive Substances: Current Topics in Behavioral Neuroscience*. Springer-Verlag; New York: 2016. 1–17.
- Kiyatkin EA, Kim AH, Wakabayashi KT, Baumann MH, Shaham Y. Effects of social interaction and warm ambient temperature on brain hyperthermia induced by the designer drugs methylone and MDPV. *Neuropsychopharmacology*. 2015; 40:436–445. [PubMed: 25074640]
- LeBlanc AE, Cappell H. Attenuation of punishing effects of morphine and amphetamine by chronic prior treatment. *J Comp Physiol Psychol*. 1974; 87:691–698. [PubMed: 4473464]
- Le Foll B, Goldberg SR. Nicotine induces conditioned place preferences over a large range of doses in rats. *Psychopharmacology*. 2004; 178:481–492. [PubMed: 15765262]
- Lin JY, Arthurs J, Reilly S. Conditioned taste aversions: from poisons to pain to drugs of abuse. *Psychon Bull Rev*. 2016; 1–17. [PubMed: 26084879]
- Marinetti LJ, Antonides HM. Analysis of synthetic cathinones commonly found in bath salts in human performance and postmortem toxicology: method development, drug distribution and interpretation of results. *J Anal Toxicol*. 2013; 37:135–146. [PubMed: 23361867]
- Marusich JA, Antonazzo KR, Wiley JL, Blough BE, Partilla JS, Baumann MH. Pharmacology of novel synthetic stimulants structurally related to the “bath salts” constituent 3,4-methylenedioxypyrovalerone (MDPV). *Neuropharmacology*. 2014; 87:206–213. [PubMed: 24594476]
- Marusich JA, Lefever TW, Blough BE, Thomas BF, Wiley JL. Pharmacological effects of methamphetamine and α -PVP vapor and injection. *Neurotoxicology*. 2016; 55:83–91. [PubMed: 27237056]
- Meltzer PC, Butler D, Deschamps JR, Madras BK. 1-(4-methylphenyl)-2-pyrrolidin-1-yl-pentan-1-one (pyrovalerone) analogues: a promising class of monoamine uptake inhibitors. *J Med Chem*. 2006; 49:1420–1432. [PubMed: 16480278]
- Merluzzi AP, Hurwitz ZE, Briscione MA, Cobuzzi JL, Wetzell B, Rice KC, Riley AL. Age-dependent MDPV-induced taste aversions and thermoregulation in adolescent and adult rats. *Dev Psychobiol*. 2013; 56:943–954. [PubMed: 24122728]
- National Research Council. Guidelines for the care and use of mammals in neuroscience and behavioral research. National Academy; Washington, D.C: 2003.
- National Research Council, U.S. Guide for the care and use of laboratory animals. National Academy; Washington, D.C: 2011.
- Patel NB. Mechanism of action of cathinone: the active ingredient of khat (*catha edulis*). *East Afr Med J*. 2000; 77:329–332. [PubMed: 12858935]
- Pechlivanova D, Tchekalarova J, Nikolov R, Yakimova K. Dose-dependent effects of caffeine on behavior and thermoregulation in a chronic unpredictable stress model of depression in rats. *Behav Brain Res*. 2010; 209:205–211. [PubMed: 20122970]
- Pomfrey RL, Bostwick TA, Wetzell BB, Riley AL. Adolescent nicotine exposure fails to impact cocaine reward, aversion and self-administration in adult male rats. *Pharmacol Biochem Behav*. 2015; 137:30–37. [PubMed: 26255152]

- Revusky S, Garcia J. Learned associations over long delays. *Psychol Learn Motiv.* 1970; 4:1–84.
- Riley AL, Davis CM, Roma PG. Strain differences in taste aversion learning: implications for animal models of drug abuse. In: Reilly SS, Todd R, editors *Conditioned Taste Aversion: Behavioral and Neural Processes*. Oxford University Press; New York: 2009. 226–261.
- Riley AL, Simpson GR. The attenuating effects of drug preexposure on taste aversion conditioning: generality, experimental parameters, underlying mechanisms and implications for drug use and abuse. In: Mowrer RR, Klein SB, editors *Contemporary Learning Theory. 2*. Lawrence Erlbaum Associates; Hillsdale New Jersey: 2001. 505–559.
- Riley AL, Tuck DL. Conditioned food aversions: a bibliography. *Ann NY Acad Sci.* 1985; 443:381–437. [PubMed: 3893270]
- Ross EA, Reisfield GM, Watson MC, Chronister CW, Goldberger BA. Psychoactive “bath salts” intoxication with methylenedioxypyrovalerone. *Am J Med.* 2012; 125:854–858. [PubMed: 22682791]
- Rozin P, Kalat JW. Specific hungers and poison avoidance as adaptive specializations of learning. *Psychol Rev.* 1971; 78:459–486. [PubMed: 4941414]
- Sañudo-Peña MC, Tsou K, Delay ER, Hohman AG, Force M, Walker JM. Endogenous cannabinoids as an aversive or counter-rewarding system in the rat. *Neurosci Lett.* 1997; 223:125–128. [PubMed: 9089689]
- Schindler CW, Thorndike EB, Goldberg SR, Lehner KR, Cozzi NV, Brandt SD, Baumann MH. Reinforcing and neurochemical effects of the “bath salts” constituents 3,4-methylenedioxypyrovalerone (MDPV) and 3,4-methylenedioxy-N-methylcathinone (methylone) in male rats. *Psychopharmacology.* 2015; 233:1981–1990. [PubMed: 26319160]
- Serafine KM, Briscione MA, Riley AL. The effects of haloperidol on cocaine-induced conditioned taste aversions. *Physiol Behav.* 2011; 105:1161–1167. [PubMed: 22212241]
- Serafine KM, Briscione MA, Rice KC, Riley AL. Dopamine mediates cocaine-induced conditioned taste aversions as demonstrated with cross-drug preexposure to GBR 12909. *Pharmacol Biochem Behav.* 2012; 102:269–274. [PubMed: 22579912]
- Serafine KM, Riley AL. Preexposure to cocaine attenuates aversions induced by both cocaine and fluoxetine: implications for the basis of cocaine-induced conditioned taste aversions. *Pharmacol Biochem Behav.* 2010; 95:230–234. [PubMed: 20122955]
- Sherman JE, Pickman C, Rice A, Liebeskind JC, Holman EW. Rewarding and aversive effects of morphine: Temporal and pharmacological properties. *Pharmacol Biochem Behav.* 1980; 13:501–505. [PubMed: 7433482]
- Shoaib M, Gommans J, Morley A, Stolerman IP, Grailhe R, Changeux JP. The role of nicotinic receptor beta-2 subunits in nicotine discrimination and conditioned taste aversion. *Neuropharmacology.* 2002; 42:530–539. [PubMed: 11955523]
- Simmler L, Buser T, Donzelli M, Schramm Y, Dieu LH, Huwyler J, Chaboz S, Hoener M, Liechti M. Pharmacological characterization of designer cathinones *in vitro*. *Br J Pharmacol.* 2013; 168:458–470. [PubMed: 22897747]
- Simpson GR, Riley AL. Morphine preexposure facilitates morphine place preference and attenuates morphine taste aversion. *Pharmacol Biochem Behav.* 2005; 80:471–479. [PubMed: 15740790]
- Stolerman IP. Cue location does not account for all selective associations in food aversion learning. *Q J Exp Psychol B.* 1985; 37:181–185. [PubMed: 4011949]
- Turenne SD, Miles C, Parker LA, Siegel S. Individual differences in reactivity to the rewarding/aversive properties of drugs: Assessment by taste and place conditioning. *Pharmacol Biochem Behav.* 1996; 53:511–516. [PubMed: 8866948]
- Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Prog Neurobiol.* 1998; 56:613–672. [PubMed: 9871940]
- Tzschentke TM. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addict Biol.* 2007; 12:227–462. [PubMed: 17678505]
- Valente MJ, Guedes de Pinho P, de Lourdes Bastos M, Carvalho F, Carvalho M. Khat and synthetic cathinones: a review. *Arch Toxicol.* 2014; 88:15–45. [PubMed: 24317389]

- Valjent E, Maldonado R. A behavioural model to reveal place preference to Δ^9 -tetrahydrocannabinol in mice. *Psychopharmacology*. 2000; 147:436–438. [PubMed: 10672638]
- Verendeve A, Riley AL. Relationship between the rewarding and aversive effects of morphine and amphetamine in individual subjects. *Learn Behav*. 2011; 39:399–408. [PubMed: 21710342]
- Verendeve A, Riley AL. Conditioned taste aversion and drugs of abuse: History and interpretation. *Neurosci Biobehav Rev*. 2012; 36:2193–2205. [PubMed: 22921283]
- Vishwanath JM, Desko AG, Riley AL. Caffeine-induced taste aversions in Lewis and Fischer rat strains: Differential sensitivity to the aversive effects of drugs. *Pharmacol Biochem Behav*. 2011; 100:66–72. [PubMed: 21810440]
- Wang YC, Huang ACW, Hsiao S. Paradoxical simultaneous occurrence of amphetamine-induced conditioned taste aversion and conditioned place preference with the same single drug injection: A new “pre- and post-association” experimental paradigm. *Pharmacol Biochem Behav*. 2010; 95:80–87. [PubMed: 20026166]
- Watterson LR, Burrows BT, Hernandez RD, Moore KN, Grabenauer M, Marusich JA, Olive MF. Effects of α -pyrrolidinovalerophenone (α -PVP) and 4-methylethcathinone (4-MEC), two synthetic cathinones commonly found in second-generation “bath salts”, on ICSS thresholds in rats. *Int J Neuropsychopharmacology*. 2014; 18:1–7.
- Wise RA, Yokel RA, DeWit H. Both positive reinforcement and conditioned aversion from amphetamine and from apomorphine in rats. *Science*. 1976; 191:1273–1275. [PubMed: 1257748]
- Woloshchuk CJ, Nelson KH, Rice KC, Riley AL. Effects of 3,4-methylenedioxypyrovalerone (MDPV) pre-exposure on the aversive effects of MDPV, cocaine and lithium chloride: Implications for abuse vulnerability. *Drug Alcohol Depend*. 2016; 167:121–127. [PubMed: 27520883]
- Xu P, Qiu Y, Zhang Y, Bai Y, Xu P, Liu Y, Kim JH, Shen HW. The effects of 4-methylethcathinone on conditioned place preference, locomotor sensitization, and anxiety-like behavior: a comparison with methamphetamine. *Int J Neuropsychopharmacol*. 2016; 19:1–7.
- Zimmer A, Valjent E, König M, Zimmer AM, Robledo P, Hahn H, Valverde O, Maldonado R. Absence of Δ^9 -tetrahydrocannabinol dysphoric effects in dynorphin-deficient mice. *J Neurosci*. 2001; 21:9499–9505. [PubMed: 11717384]

Highlights

- α -PVP induced significant dose-dependent taste aversions.
- α -PVP produced significant conditioned place preferences.
- α -PVP induced dose-dependent hyperthermia.
- There was no relationship between taste avoidance and hyperthermia.

Figure 1. Mean (+/- SEM) saccharin consumption (ml) over Trials 1–4 for groups injected with a-PVP or vehicle following saccharin consumption. *Significantly different from Groups Vehicle, 0.3 and 1.0. #Significantly different from Trial 1. xSignificantly different from Trial 2.

Figure 2.

Mean ($\pm$ SEM) water consumption (ml) over Recovery Days 1–4 for groups injected with vehicle following water consumption. *Significantly different from Groups Vehicle, 0.3 and 1.0. #Significantly different from Trial 1. xSignificantly different from Trial 2.

Figure 3.

Mean ($\pm$ SEM) percent saccharin consumed for each group during the final two bottle test.

*Significant difference from Groups Vehicle and 0.3.

Figure 4. Mean ($\pm$ SEM) percent time spent on the drug-paired side for each group at Pre-Test (white bars) and Post-Test (black bars). *Significant change from Pre-test to Post-test.

Figure 5. Mean (+/- SEM) temperature in °C for each group at pre-injection and 30, 60, 120 and 180 minutes post injection. *Significantly different from vehicle. #Significantly different from pre-injection. xSignificantly different from 30 minute.